# Strategy for AMR Surveillance in the Environment (2025-2027)

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# Table of Contents

# List of Tables



# List of Figures

# List of Abbreviations

| | |
|---|---|
| ADP | Annual Development Programme (ADP) |
| AI | Artificial Intelligence |
| AMR | Antimicrobial Resistance |
| AMU | Antimicrobial Use |
| APHA | American Public Health Association |
| ARB | antibiotic-resistant bacteria |
| ARGs | Antibiotic Resistance Genes |
| ASP | Antibiotic Stewardship Programs |
| AST | Antimicrobial Susceptibility Testing |
| BPPL | Bacterial Priority Pathogens List |
| CDA | Capital Development Authority |
| CLSI | Clinical Laboratory Standards Institute |
| DHIS | District Health Information Software |
| DO | Dissolved Oxygen (DO) |
| DRAP | Drug Regulatory Authority of Pakistan |
| EPA | Environmental Protection Agencies |
| EQA | External Quality Assurance |
| ESBL-Ec | Extended-Spectrum Beta-Lactamase-Producing Escherichia Coli |
| ETPs | Effluent Treatment Plants |
| FAO | Food and Agriculture Organization |
| FDCL | Food and Drug Control Laboratory |
| GC-MS | Gas Chromatography-Mass Spectrometry |
| GIS | Geographic Information Systems |
| GLASS | Global Antimicrobial Resistance Surveillance System |
| HEC | Higher Education Commission |
| HRMS | High-Resolution Mass Spectrometry |
| IPC | Infection Prevention and Control |
| IQC | Internal Quality Control |
| KPIs | Key Performance Indicators (KPIs) |
| LC-MS/MS | Liquid Chromatography-Mass Spectrometry |
| LIMS | Laboratory Information Management System |
| M&E | Monitoring and Evaluation |
| MDR | Multidrug-Resistant |
| MGEs | Mobile Genetic Elements |
| ML | Machine Learning |
| MoCC | Ministry of Climate Change and Environmental Coordination |
| MoIB | Ministry of Information and Broadcasting |
| MoIP | Ministry of Industries and Production |
| MoNFSR | Ministry of National Food Security and Research |
| MoNHSRC | Ministry of National Health Services, Regulations and Coordination |
| MoPDSI | Ministry of Planning Development and Special Initiatives |

| | |
|---|---|
| MoST | Ministry of Science and Technology |
| MoU | Memorandums of Understanding |
| MoWR | Ministry of Water Resources |
| NAP | Pakistan's National Action Plan |
| NGO | Non-Governmental Organizations |
| NGS | Next-Generation Sequencing |
| NIH | National Institutes of Health |
| NRL | National Reference Laboratory |
| Pak-EPA | Pakistan Environmental Protection Agency |
| PARB | Punjab Agricultural Research Board |
| PARC | Pakistan Agricultural Research Council |
| PCR | Polymerase Chain Reaction |
| PCRWR | Pakistan Council of Research in Water Resources |
| PCSIR | Pakistan Council of Scientific and Industrial Research |
| PHED | Public Health Engineering Departments |
| POPs | Persistent Organic Pollutants |
| PSDP | Public Sector Development Programme |
| PT | Proficiency Testing |
| QC | Quality Control |
| QMS | Quality Management System |
| qPCR | Quantitative Polymerase Chain Reaction |
| SDGs | Sustainable Development Goals |
| SOPs | Standard Operating Procedures |
| STPs | Sewage Treatment Plants |
| TWG | Technical Working Group |
| UNEP | United Nations Environment Program |
| UNICEF | United Nations International Children's Emergency Fund |
| USAID | United States Agency for International Development |
| WASA | Water and Sanitation Authorities |
| WASH | Water, Sanitation and Hygiene |
| WGS | Whole Genome Sequencing |
| WHA | World Health Assembly |
| WHO | World Health Organization |
| WOAH | World Organization for Animal Health |

# Acknowledgement

## Collaborating Partners

Fleming Fund Country Grant, DAI-Pakistan,
Ministry of National Health Services, Regulations and Coordination
Ministry of Climate Change and Environmental Coordination,
National Agricultural Research Centre (NARC),
Ministry of National Food Security and Research,
Ministry of Water Resources

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# Foreword

Antimicrobial Resistance (AMR) presents a formidable challenge to global public health, with its environmental dimensions demanding urgent and sustained attention. In Pakistan, the growing prevalence of AMR in environmental settings—spanning water systems, agricultural runoff, and industrial effluents—underscores the need for a systematic and evidence-based approach to mitigate its spread. Addressing these environmental pathways is essential, as they contribute significantly to the persistence and transmission of resistant pathogens, posing risks to human, animal, and ecosystem health.

Recognizing the critical nature of this issue, the Strategy for AMR Surveillance in the Environment (2025–2027) has been developed as a landmark initiative to strengthen monitoring frameworks and mitigate the spread of AMR through environmental routes. This strategy represents a pivotal step in Pakistan's ongoing efforts to tackle AMR comprehensively, ensuring that environmental factors are given due priority alongside clinical and veterinary aspects. Aligned with global frameworks such as the United Nations Environment Programme (UNEP) Framework on AMR, the World Health Organization (WHO) Global Action Plan on AMR, and Pakistan's National Action Plan on AMR, this initiative reinforces the country's commitment to international standards and best practices in AMR management. It also contributes to the achievement of Sustainable Development Goals (SDGs), particularly SDG 3 (Good Health and Well-being), SDG 6 (Clean Water and Sanitation), and SDG 12 (Responsible Consumption and Production), reflecting Pakistan's dedication to sustainable and effective AMR mitigation.

The Ministry of National Health Services, Regulations, and Coordination (MoNHSRC) proudly supports this initiative, working in close collaboration with key national and international stakeholders. This includes Fleming Fund, DAI, Pakistan Council of Research in Water Resources (PCRWR), academia, the private sector, and civil society organizations, all of whom play a vital role in ensuring the strategy's success. The collective expertise and commitment of these partners highlight the importance of a multi-sectoral approach in combating AMR at both policy and operational levels.

This strategy is more than a roadmap—it is a collective commitment to safeguarding public health, protecting vital ecosystems, and ensuring the sustainable use of antimicrobial agents in Pakistan. Its successful implementation will depend on strong governance, enhanced laboratory capacities, robust data-sharing mechanisms, and active stakeholder engagement across all sectors. I commend the dedicated efforts of all those involved in the formulation of this strategy and emphasize the urgency of its timely and effective implementation. The challenge of AMR is vast, but through collaboration, innovation, and sustained commitment, we can strengthen our defense against this global health threat. Let us work together to build a healthier and more sustainable future for generations to come.

**Mirza Nasir Uddin Mashhood Ahmad**

**Special Secretary**
Ministry of National Health Services,
Regulations, and Coordination
Islamabad, Pakistan

# Prologue

Antimicrobial Resistance (AMR) is an escalating global health challenge with profound implications for public health, economic stability, and environmental sustainability. In Pakistan, widespread antibiotic misuse—both in healthcare and agriculture—coupled with unregulated disposal into the environment, exacerbates this crisis. The environment plays a key role as both a reservoir and transmission pathway for resistant bacteria and genes, making it a critical focus in the fight against AMR.

The Strategy for AMR surveillance in the environment (2025–2027) is a significant step toward addressing AMR's environmental impact. Anchored in the One Health approach, it integrates human, animal, and environmental health, aligning with global frameworks like the WHO Global Action Plan and UNEP's environmental AMR guidelines. This strategy emphasizes evidence-based interventions, such as robust surveillance, regulatory reforms, public awareness, and capacity building.

This achievement is the result of collective efforts from key stakeholders, including the Fleming Fund, Health Security Partners, UK AID, the Ministry of National Health Services, Regulations, and Coordination (MoNHSRC), and the Pakistan Council of Research in Water Resources (PCRWR). Their unwavering support has been instrumental in developing this strategy. We also extend our gratitude to Development Alternatives Inc. (DAI) for their facilitation in advancing this initiative.

The strategy introduces a comprehensive framework for AMR surveillance in the environment, aiming to establish baseline AMR data from samples derived from the environment, strengthen integrated surveillance by including AMR data components reflective of the environment, and propose an enhanced analysis of antimicrobial resistant-bacteria recovered from the environment using molecular approaches to better understand the epidemiology of AMR. Formulated with input from provincial departments, academic institutions, farmers, industrialists, and donor agencies, this strategy is poised to make a substantial impact on environmental AMR surveillance across Pakistan.

I congratulate all team members involved in achieving this milestone. We look forward to the successful implementation of this strategy and the strengthening of AMR laboratories for environmental surveillance across the country. Let us unite in our efforts to combat AMR, protecting public health, preserving ecosystems, and securing a sustainable future.

**Dr Hifza Rasheed**
Director General,
Pakistan Council of Research in Water Resources,
Islamabad

## Message from Team Lead, FF Country Grant Pakistan

At Development Alternatives Inc. (DAI), we are honored to support the development of Pakistan's Strategy for AMR Surveillance in the Environment, a vital initiative in combating one of the most pressing global health challenges: Antimicrobial Resistance (AMR). AMR transcends borders and disciplines, necessitating innovative and integrated approaches that address its human, animal, and environmental dimensions.

The Fleming Fund, established by the UK in response to the UK AMR review and the WHO Global Action Plan on AMR, has been instrumental since 2019 in supporting Pakistan's comprehensive efforts against AMR across Human, Veterinary, and Environmental sectors. This AMR Environmental Surveillance Strategy, developed as part of this collaboration, aims to aid authorities in managing and controlling antibiotic use in the environment, significantly impacting both human and veterinary health outcomes.

This strategy embodies a shared commitment to tackling AMR through the One Health framework, aligning with the Pakistan's National Action Plan on AMR (2.0), leveraging cross-sectoral collaboration, robust evidence, and international best practices. Central to the strategy is its robust AMR Environmental Surveillance Framework, designed to systematically monitor and analyze environmental data. This framework will help identify AMR hotspots, providing critical insights into the environmental reservoirs of resistance. These findings will be pivotal in informing and supporting the implementation of national policies aimed at mitigating AMR risks. By focusing on AMR surveillance of environment, policy reforms, capacity building, and public awareness, it seeks to mitigate AMR risks while promoting sustainable development.

The development of this strategy has been a collaborative effort, made possible through the invaluable contributions of our partners. We extend our deepest appreciation to the Fleming Fund for their critical funding support, Health Security Partners and UKAID for their guidance and resources, the Ministry of National Health Services, Regulations and Coordination Pakistan for their leadership, and the Pakistan Council of Research in Water Resources (PCRWR) for their scientific expertise and unwavering commitment. DAI is steadfast in its mission to align Pakistan's national strategies with global frameworks, such as the WHO Global Action Plan on AMR. We are committed to fostering partnerships and delivering impactful solutions that reduce the burden of AMR on communities, ecosystems, and economies. The Fleming Fund Country Grant in Pakistan remains committed to supporting the people and government of Pakistan in this crucial fight against AMR.

**Dr. Qadeer Ahsan**
Team Lead
Fleming Fund Country Grant Pakistan
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# Executive Summary

Antimicrobial Resistance (AMR) is a pressing global health threat, intensified by environmental contamination and the indiscriminate use of antibiotics across multiple sectors. Water bodies, soil, and wastewater act as reservoirs and conduits for resistant bacteria and genes, facilitating their spread and amplifying risks to both public health and ecological balance.

In Pakistan, national policies have largely overlooked the environmental aspects of AMR. However, comprehensive environmental surveillance, aligned with international strategies and the One Health approach, can effectively track resistance, assess mitigation efforts, and support waste management and policy enforcement. To address this, a Strategy for AMR Surveillance in the Environment (2025–2027) has been developed through a consultative and inclusive process.

The alignment of Pakistan's AMR Environmental Surveillance Strategy with NAP 2.0 (2024-2028) is a crucial step in establishing the country's first comprehensive framework for AMR surveillance. Key stakeholders, including government agencies, academia, international organizations, the private sector, and civil society, have been engaged in structured discussions and workshops to ensure the strategy is both inclusive and actionable.

This document outlines a phased, three-year roadmap to strengthen Pakistan's environmental monitoring infrastructure, enhance data management systems, and promote alignment with WHO's Global Action Plan on AMR. The strategy focuses on capacity building, public awareness, and multi-sectoral collaboration to mitigate the spread of AMR and contribute to achieving the United Nations Sustainable Development Goals (SDGs). Additionally, a proposed five-year framework will align with the One Health concept, targeting critical surveillance areas such as antibiotic-resistant bacteria, genes, and high-risk pathways. This framework will utilize WHO guidelines and Key Performance Indicators (KPIs) to track progress and ensure effective policy implementation and capacity building in the fight against AMR.

A pilot study on AMR surveillance in water and wastewater sources is also included in the strategy. This study aims to bridge data and capacity gaps, strengthen surveillance, monitor pathogens, assess resistance patterns, and inform policy reforms to reduce AMR risks and improve water quality management. The strategy also identifies gaps in Pakistan's AMR policy landscape and proposes KPIs to monitor progress, including targets for reducing antibiotic residues, detecting resistant bacteria, and achieving full coverage in high-risk areas.

The strategy calls for collaborative action from all stakeholders—government agencies, private sector actors, academia, and civil society—to safeguard public health and the environment. It reflects a shared commitment to combating AMR, ensuring sustainable development, and building resilience against future public health threats.

## Glossary of Terms

**Antimicrobial Resistance**: The ability of microorganisms to resist the effects of medication that once could successfully treat the microorganism.

**Antimicrobial Resistant Bacteria**: Bacteria that have developed resistance to antibiotics.

**Antibiotic Resistance Genes**: Genes in bacteria that confer resistance to antibiotics.

**Antimicrobial Susceptibility Testing**: A procedure to determine the effectiveness of antibiotics on a microorganism.

**Extended-Spectrum Beta-Lactamase**: Enzymes produced by bacteria that confer resistance to cephalosporins and monobactams.

**One Health Approach**: A collaborative approach recognizing the interconnectedness of human, animal, and environmental health in addressing health challenges like AMR.

**Laboratory Information Management System**: Software that manages samples, associated data, and laboratory workflows.

**Geographic Information System**: A framework for gathering, managing, and analyzing spatial and geographic data.

**Polymerase Chain Reaction**: A technique used to amplify small segments of DNA.

**External Quality Assurance**: Procedures ensuring laboratories maintain high standards of accuracy in their testing.

**Quality Assurance**: Systems ensuring the quality and reliability of laboratory results.

**World Health Organization**: A specialized agency of the United Nations responsible for international public health.

**Food and Agriculture Organization**: A specialized agency of the United Nations leading international efforts to defeat hunger.

**National Action Plan**: A strategic plan for combating antimicrobial resistance at the national level.

**Sewage Treatment Plants**: Facilities designed to treat wastewater before releasing it into the environment.

**VITEK 2**: An automated system for identification of bacteria and antimicrobial susceptibility testing.

**Metagenomics**: The study of genetic material recovered directly from environmental samples.

**Bacterial Priority Pathogens List**: A WHO list prioritizing bacteria that pose the greatest threat to human health due to their resistance to antibiotics.

**Mobile Genetic Elements**: DNA segments that can move between genomes, often carrying antibiotic resistance genes.

# 1 Introduction

## 1.1 Background

Antimicrobials, including antibiotics, antivirals, antifungals, and anti-parasitics, are crucial for preventing and treating infections in humans, animals, and plants. However, Antimicrobial Resistance (AMR) occurs when microorganisms no longer respond to these medicines, making infections harder to treat and increasing the risk of disease spread, severe illness, and death. In 2015, the World Health Assembly adopted a global action plan on AMR with objectives aimed at improving awareness, strengthening research, reducing infections, optimizing antimicrobial use, and investing in new treatments.

In Pakistan, AMR is driven by demographic pressures, environmental degradation, inadequate healthcare delivery, agricultural practices, and regulatory gaps. The overuse of antibiotics in healthcare and agriculture, compounded by environmental contamination, contributes significantly to AMR's rise. Key factors include a large population (241 million), poor sanitation, limited healthcare resources, and high antibiotic use in agriculture. A significant portion of the population lacks clean water and sanitation, further exacerbating AMR through waterborne diseases. Additionally, weak legislation, poor enforcement, and limited surveillance systems hinder effective AMR monitoring and control.

The environment plays a crucial role in AMR as both a reservoir and transmission pathway. Environmental monitoring is limited, preventing a full understanding of AMR dynamics. Environmental degradation, driven by climate change, pollution, and biodiversity loss, further exacerbates AMR by altering microbial diversity and promoting resistance. This has serious implications for achieving Sustainable Development Goals (SDGs), especially in health, poverty, food security, and economics. The United Nations Environment Assembly's 2017 Resolution stressed the need to understand the links between the environment and AMR. The One Health Approach, which integrates human, animal, and environmental health, is essential in combating AMR. Comprehensive environmental AMR surveillance is necessary for understanding the spread of resistance across environmental and clinical boundaries and implementing effective mitigation strategies.

## 1.2 Challenges and Strategic Considerations for AMR Surveillance in Environment in Pakistan

Environmental AMR is a critical issue in Pakistan, with significant challenges arising from multiple sectors due to poor regulation, inadequate infrastructure, and a lack of awareness. Below is a sector-specific breakdown of the environmental AMR challenges in Pakistan.

### 1.2.1 Healthcare Sector

Hospitals and clinics often lack effective systems for treating and disposing of medical waste, including antibiotics and other pharmaceuticals. This waste enters the environment, promoting the development of resistant bacteria. Over-prescription and self-medication with antibiotics are rampant, leading to increased antibiotic residues in wastewater.

### 1.2.2 Agricultural Sector

Pakistan has a diversified economic base with the agriculture sector, contributing 24% in GDP and 37.4% in employment (Pakistan Economic Survey 2023-24, 2024). The agricultural sector also contributes significantly to AMR contamination through the widespread use of antibiotics in livestock and crops. Pakistan is among the top 10 countries that are producing food animals through modern farming practices and rely on antibiotics as growth promoters and for disease prevention (Shafiq et al., 2022). Antibiotics are also widely used in poultry, dairy, and aquaculture, both for therapeutic purposes and as growth promoters. This contributes to antibiotic residues in manure, which is often used as fertilizer, contaminating soil and water. Some pesticides contain antimicrobial agents, further driving resistance in soil microbiomes. However, there is unfortunately no estimation of annual antibiotic use in food-producing animals in Pakistan.

### 1.2.3 Industrial Sector

The pharmaceutical market in Pakistan, valued at Rs. 748 billion in 2023 is growing at a 5-year CAGR of 15.3% (The Institute of Chartered Accountants of Pakistan, 2024). Local and national companies dominate over two-thirds of the market, with around 650 companies, including multinationals, contributing 1% to the GDP annually. Approximately 11,000 drugs are actively marketed in Pakistan, alongside a significant segment of OTC products like multivitamins and cold remedies. Pharmaceutical manufacturing facilities often discharge untreated effluents with high antibiotic concentrations, while the textile industry releases wastewater with biocides, both contributing to environmental AMR.

### 1.2.4 Water and Sanitation Sector

Inadequate wastewater treatment facilities result in untreated sewage carrying antibiotic residues and resistant bacteria entering rivers, lakes, and groundwater. Poor water management and infrastructure allow resistant bacteria to spread through drinking water systems. Pakistan's environmental challenges, such as water contamination, poor waste management, and inadequate sanitation, exacerbate the spread of AMR. With 61% of water sources deemed unsafe for drinking (Hifza et al., 2021) and 32% of the population lacking access to sanitation (UNICEF, 2024), environmental reservoirs become key pathways for antibiotic-resistant bacteria (ARB) transmission. Individuals often dispose of expired or unused antibiotics improperly, leading to contamination of water and soil. Lack of access to proper sanitation facilities facilitates the spread of resistant pathogens in communities.

AMR surveillance in Pakistan faces significant challenges, primarily due to a lack of focus on environmental reservoirs such as water bodies, soil, and wastewater. Surveillance efforts are mostly concentrated on human and animal health, leaving critical environmental sources and transmission pathways unmonitored. Moreover, limited resources, specialized laboratories, and trained personnel further hinder effective environmental AMR management. The absence of integrated data systems across human, animal, and environmental sectors leads to fragmented data collection, obscuring the full scope of AMR dynamics. Studies showed resistance levels for common infections in Pakistan range from 20% to 40%, highlighting the urgency for comprehensive AMR interventions (Mirha et al., 2024). To address these gaps, Pakistan needs to strengthen environmental monitoring infrastructure, develop integrated data systems, implement stricter regulations, and enhance research and awareness efforts targeting environmental AMR. Integrating environmental considerations into AMR policies and enhancing institutional capacity for surveillance are essential steps toward combating the growing AMR crisis in Pakistan.

## 1.3 What encompasses 'The Environment' in AMR Surveillance in Environment Framework?

In an environmental surveillance framework, "the environment" refers to interconnected systems and compartments where AMR can emerge, persist, and spread. These include water bodies (such as rivers, lakes, and sediments), sewage, soil, air, wildlife, and agricultural ecosystems, all of which can harbour antimicrobials, antimicrobial resistant microorganisms, and antibiotic resistance genes as depicted in *Figure-1*. This indicates that antimicrobial pollution primarily originates from five key sources including municipal waste and effluents; pharmaceutical manufacturing; healthcare facilities; animal, and crop production and the disposal of manure or by products of crops contaminated with antimicrobial residues and antimicrobial resistant bacteria/genetic elements. These pollutants introduce antibiotic-resistant bacteria (ARB), ARGs, and mobile genetic elements (MGEs) into environmental systems. Therefore, environment acts as both a recipient and reservoir for AMR, with contamination spreading diffusely from multiple sources. Environmental AMR surveillance provides insights into monitoring resistant organisms and genes entering the environment from sources like sewage discharges and understanding exposure pathways affecting humans, animals, and ecosystems through environmental contact.

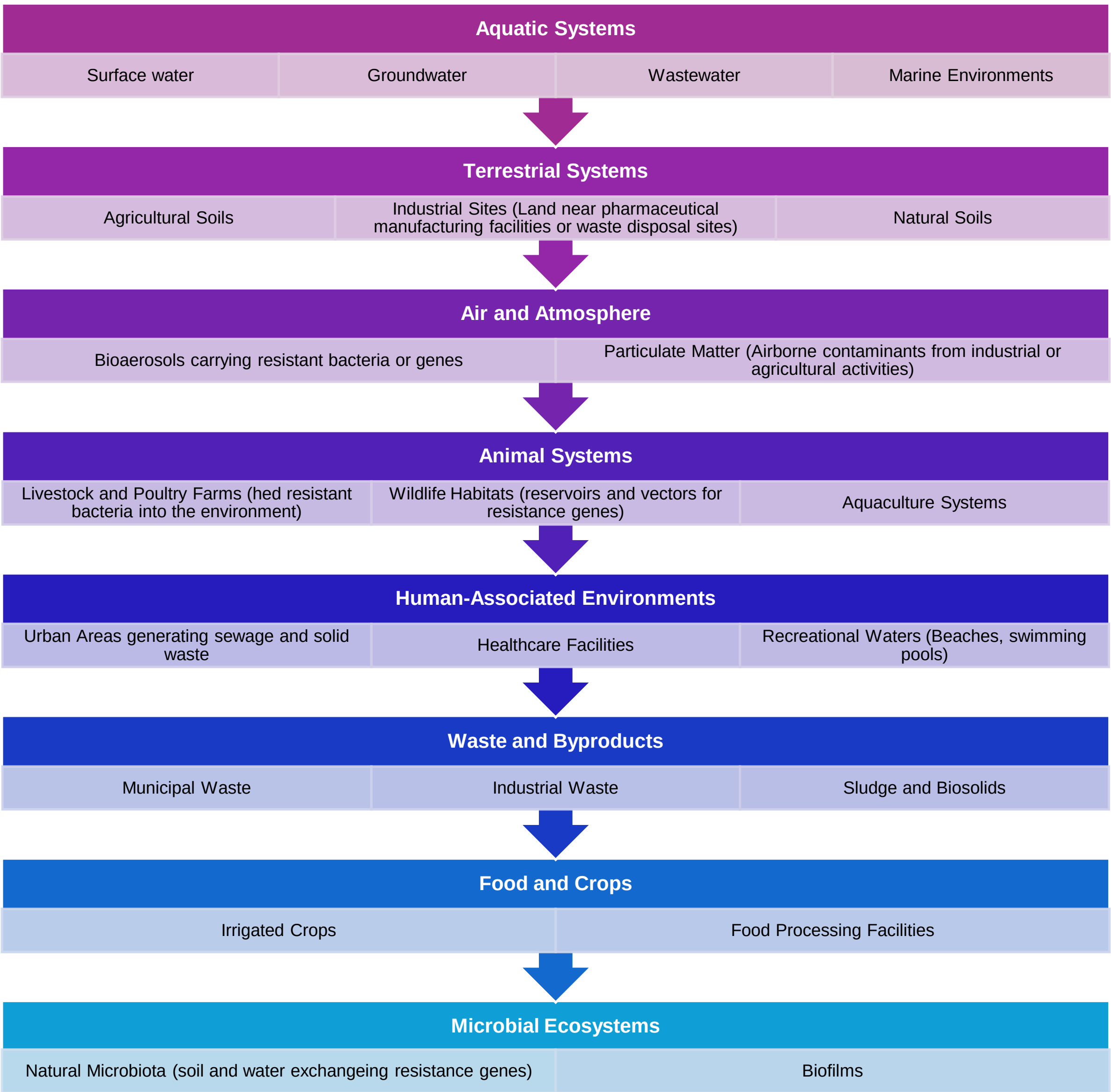


*Figure 1: Environmental compartments harbouring recipient and reservoir for AMR*

## 1.4 Rationale for Strategy for AMR surveillance in the environment

AMR is a critical global health concern, with environmental pathways playing a significant role in its emergence and spread. Recognizing this, international organizations like the EU, UN, and WHO emphasize the need for effective environmental surveillance systems to monitor AMR across water, wastewater, soil, air, and wildlife. These systems help understand how antimicrobials and resistant bacteria from human and animal sources contribute to AMR-related health issues when introduced into the environment.

In Pakistan, this issue is urgent, yet national policies such as the National Water Policy (2018), Climate Change Policy (2012, updated 2021), and various provincial regulations largely overlook AMR's environmental dimensions. Comprehensive environmental AMR surveillance could enable Pakistan to track resistance evolution, assess mitigation measures, and align with international strategies like the EU One Health Action Plan, UK 5-Year AMR Action Plan, and the US National Action Plan for Combating Antibiotic-Resistant Bacteria. This approach supports the Sustainable Development Goals (SDGs), especially SDG 3 (Good Health), SDG 6 (Clean Water), SDG 12 (Responsible Consumption), and SDG 13 (Climate Action).

The One Health Approach, endorsed by WHO, FAO, WOAH, and UNEP, integrates environmental, human, and animal health in AMR action plans through:

1. **Environmental Surveillance:** Monitoring antibiotic residues, resistance genes, and AMR hotspots in soil, water, and agricultural runoff.
2. **Waste Management:** Managing pharmaceutical, agricultural, and healthcare waste to prevent environmental contamination with antimicrobials and resistant bacteria.
3. **Policy and Regulation:** Enforcing environmental policies to control antimicrobial discharge into the environment.

An Environmental AMR Surveillance Strategy for Pakistan would involve systematic monitoring of water bodies, sewage, and agricultural environments, focusing on hotspots like healthcare and food processing waste. Aligning with international frameworks like the WHO Global Action Plan on AMR is crucial for designing data-driven interventions. Incorporating AMR monitoring into existing policies enhances their effectiveness in managing water, agriculture, climate change, and public health. This strategy not only strengthens Pakistan's response to AMR but also contributes to global efforts, safeguarding public health and supporting sustainable development.

## 1.5 Scope of the Strategy for AMR surveillance in the environment

The Environmental Antimicrobial Resistance (AMR) Surveillance Strategy aims to monitor and mitigate the spread of AMR within key environmental compartments, such as water, soil, air, and food systems. This strategy employs an integrated One Health approach, creating a cohesive national framework that links human, animal, and environmental AMR surveillance. It aligns with global initiatives such as the Global Action Plan on Antimicrobial Resistance (WHO), the One Health Global Leaders Group on Antimicrobial Resistance (GLG-AMR: WHO, FAO, WOAH), the Codex Alimentarius (FAO/WHO), and the Quadripartite Collaboration on AMR (FAO, UNEP, WHO, WOAH). By fostering collaboration, enhancing monitoring, and strengthening prevention and control measures, the strategy will support the policy development and governance frameworks, ultimately safeguarding both human and environmental health and contributing to global health security. Additionally, it aims to

establish systematic AMR surveillance in high-risk areas such as wastewater treatment facilities, livestock farms, pharmaceutical effluent discharge sites, and urban runoff-affected waters. The strategy will focus on the following specific objectives:

i. **Develop Standardized Surveillance Systems:** Develop and implement comprehensive surveillance systems across key environmental compartments (e.g., wastewater, agricultural runoff, soil) to monitor antimicrobial residues, resistant bacteria, and resistance genes, using advanced molecular techniques for data collection and evidence-based policymaking.

ii. **Identify AMR Hotspots and Transmission Pathways:** Identify high-risk areas (e.g., wastewater discharge points, agricultural fields) and trace the transmission of AMR from sources to receptors, to inform targeted interventions and improve understanding of AMR spread through environmental pathways.

iii. **Develop and Implement AMR Data Management System:** Establish an integrated data management system to collect, store, and analyze environmental AMR data, ensuring efficient sharing of information across sectors and supporting informed decision-making for AMR surveillance and management.

iv. **Support Policy Development and Regulatory Frameworks:** Utilize surveillance data to influence policy development on antimicrobial use and waste management, ensuring alignment with national and global frameworks such as the WHO Global Action Plan on AMR.

v. **Promote Research and Sustainable Solutions:** Encourage and support research into effective AMR management strategies and sustainable practices, facilitating long-term surveillance to mitigate environmental AMR risks.

Pakistan's Environmental AMR Surveillance Strategy aligns with the **National AMR Action Plan**, which emphasizes integrated surveillance, hotspot identification, and evidence-based policymaking to mitigate antimicrobial resistance. It also supports the **WHO Global Action Plan on AMR**, which calls for robust surveillance systems, improved understanding of AMR transmission through environmental sources, and multi-sectoral collaboration to combat AMR globally.

## 1.6 Strategy Development Approach (From Roadmap to Success)

All AMR stakeholders in Pakistan have concerns about the AMR implications in the country. Accordingly, there was a need to come up with clear approach of strategy framing process by involving all of them. Therefore, Environmental Strategy is developed using a qualitative approach to address antimicrobial resistance through environmental surveillance. In this regard, following methodology was adopted.

### 1.6.1 Literature Review

A thorough review of global and national policies, research studies, and frameworks on AMR and environmental surveillance was conducted. Key documents analyzed included those from the World Health Organization (WHO), the Food and Agriculture Organization (FAO), the World Organization for Animal Health (WOAH), UNEP, and Pakistan's National Action Plan (NAP) on AMR. This review provided a foundational understanding of existing knowledge and gaps in AMR environmental management.

### 1.6.2 Consultation Workshop

A one-day consultation workshop was organized on October 2, 2024 bringing together participants from national and provincial ministries, academia, MONHSRC, environmental experts, UN agencies, and other relevant stakeholders. The workshop facilitated discussions on the intersection of environmental health and AMR, identifying challenges, sharing best practices, exploring surveillance solutions and identifying the necessary consumables and equipment for effective laboratory-based environmental AMR detection.

### 1.6.3 Roundtable Discussion

A roundtable discussion was conducted with a focused group of participants, including representatives from MONHSRC, academia, provincial ministries, environmental experts, and UN agencies. This platform enabled in-depth deliberations on policy recommendations for environmental AMR surveillance under the One Health framework.

### 1.6.4 Data Collection and Analysis

Existing environmental data from Pakistan Council of Research in Water Resources (PCRWR), National Institute of Health (NIH), Pakistan Environmental Protection Agency (Pak-EPA), Provincial Environmental Protection Agencies, Pakistan Agricultural Research Council (PARC), and Public Health Engineering Departments (PHED) were reviewed. The analysis focused on identifying critical points in the environment that contribute to AMR spread, including water bodies, soil, and agricultural runoff.

This enhanced section reflects a broader collaboration, leveraging data from multiple national and provincial agencies to provide a more comprehensive understanding of environmental factors influencing AMR spread.

### 1.6.5 Case Studies

Successful examples of AMR environmental surveillance systems from countries such as Netherlands (Altorf-van der Kuil et al., 2017, Pandey et al., 2022) and Singapore (Lim et al., 2020, Poon et al., 2023, Ng and Gin, 2019) were examined. These case studies informed the development of tailored recommendations for Pakistan, ensuring the strategy is contextually relevant and effective.

Followed by the consultative a consultative process was initiated to seek opinions/feedback/ concerns of AMR stakeholders which are incorporated into this document as it is developed (*Figure-2*).

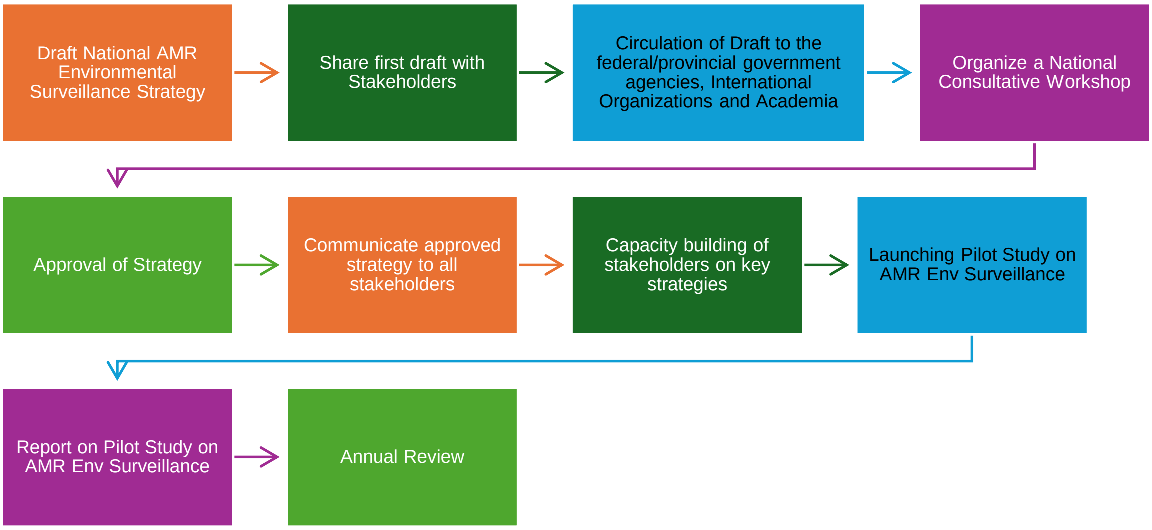


*Figure 2: Process Adapted from Framing National Strategies to Implementation*

## 1.7 Key Stakeholders Engaged in the Development of Strategy

A brief overview of role of key stakeholders contributed in the strategy development is as following:

1. **Pakistan Council of Research in Water Resources:** Key stakeholder which provided detailed situational analysis of environmental sector, current trends in monitoring and surveillance across the country, gaps and deficiencies and future requirements for AMR surveillance system. This helped to formulate the requirements and framework of AMR environmental surveillance as included in the strategy.
2. **Ministry of National Health Services, Regulations and Coordination (MoNHSRC):** Played a pivotal role in coordinating cross-sectoral efforts and ensuring alignment of the strategy with Pakistan's National Action Plan (NAP) on AMR. MoNHSRC also facilitated policy discussions and provided guidance on integrating AMR surveillance into healthcare and public health systems.
3. **Animal Husbandry and Veterinary Departments:** Highlighted the use of antimicrobials in livestock and the role of animals as reservoirs of AMR. Provided insights on antimicrobial practices in veterinary settings and contributed to developing surveillance frameworks for agricultural runoff and farm effluents.
4. **Academia:** shared research on AMR and provided insights on scientific methodologies for environmental sampling and laboratory testing.

In December 2024, the Strategy for AMR Surveillance in the Environment (2025–2027) was ratified, marking a critical milestone in Pakistan's efforts to combat antimicrobial resistance.

## 1.8 Stakeholders Role in Strategy Implementation

The successful development and implementation of an environmental AMR surveillance strategy in Pakistan demands the active involvement of a broad range of stakeholders, each playing a specific and critical role in combating antimicrobial resistance (AMR). These stakeholders come from various sectors such as public health, environmental management, agriculture, industry, and policy-making, and their coordinated efforts are essential for addressing the increasing threat of AMR. By establishing clear roles for each stakeholder, Pakistan can ensure the effective integration of environmental AMR surveillance into existing

systems and contribute to global efforts in controlling AMR. A list of identified stakeholders and their anticipated roles in the successful implementation of this strategy is outlined in *Table-1* below:

**Table 1: Key stakeholders and Anticipated Roles in Strategy Implementation**

| SN | Stakeholder | Anticipated Role |
|---|---|---|
| **1.** | Pakistan Council of Research in Water Resources (PCRWR) | Lead research and monitoring of AMR in water/wastewater sources, particularly initiating pilot study as included in this strategy |
| **2.** | Development Alternatives, Inc. (DAI) Pakistan | Provide financial and technical expertise and support for implementing AMR surveillance in environmental and public health systems. |
| **3.** | Ministry of National Health Services, Regulations and Coordination (MoNHSRC) | Oversee national AMR strategy, including environmental surveillance. Coordinate cross-sectoral efforts. |
| **4.** | Ministry of Water Resources (MoWR) | Ensure AMR monitoring is integrated into the National Water Policy. |
| **5.** | Ministry of Climate Change and Environmental Coordination (MoCC) | Integrate AMR surveillance into climate change adaptation and mitigation strategies, considering environmental health impacts. |
| **6.** | Ministry of Food Security and Research (MoFSR) | Coordinate surveillance related to AMR in agriculture and food production, ensuring food safety and sustainable agricultural practices |
| **7.** | Ministry of Planning, Development, and Special Initiatives (MoPDSI) | Oversee the integration of AMR environmental surveillance in national development plans, facilitate resource allocation, and ensure strategic planning for AMR monitoring. |
| **8.** | Ministry of Information and Broadcasting (MoIB) | Raise public awareness about AMR through media campaigns, disseminate information on AMR risks, and promote responsible antimicrobial use. |
| **9.** | Ministry of Industries and Production (MoIP) | Ensure industry compliance with regulations on antimicrobial usage and discharge; promote sustainable practices in pharmaceutical and other industries. |
| **10.** | Pakistan Environmental Protection Agency (Pak-EPA) | Regulate pollutants, antimicrobial residues, and resistant microorganisms. |
| **11.** | National Institutes of Health (NIH) | Provide technical support, including laboratory analysis (metagenomics) and research on AMR in the multisector. |
| **12.** | Public Health Engineering Departments (PHED) | Oversee water supply and sanitation systems, including monitoring AMR in drinking water and wastewater. |
| **13.** | Water and Sanitation Authorities (WASA) | Maintain water and sanitation infrastructure, focusing on AMR reduction in wastewater, acting as key points for AMR detection and mitigation |
| **14.** | Provincial Health Departments | Implement and manage local AMR surveillance programs, ensuring alignment with national standards. |
| **15.** | Federal and Provincial Irrigation Departments | Monitor AMR in agricultural water, including runoff and irrigation water. |
| **16.** | World Health Organization (WHO) | Offer technical expertise and resources for AMR strategy development, capacity building, and international collaboration. |

| SN | Stakeholder | Anticipated Role |
|---|---|---|
| **17.** | United Nations Environment Programme (UNEP) | Assist in setting global standards, frameworks, and best practices for environmental AMR surveillance. |
| **18.** | Food and Agriculture Organization (FAO) | Guide AMR surveillance in agriculture, including monitoring antibiotic use in farming and ensuring food safety. |
| **19.** | Private Sector (Pharmaceutical and Agricultural Industries) | Reduce antimicrobial release into the environment through better waste management and sustainable practices. |
| **20.** | Non-Governmental Organizations (NGOs) and Civil Society | Advocate for policy inclusion, monitor AMR surveillance implementation, and raise public awareness. |
| **21.** | Local Governments (District and Municipal Authorities) | Implement local AMR surveillance, monitor water sources, sewage, and wastewater treatment plants. |
| **22.** | Animal Husbandry and Veterinary Departments | Monitor antibiotic use in livestock; contribute to understanding the role of animals in AMR transmission. |
| **23.** | Federal Environmental Protection Agency | Oversee compliance efforts, enforce discharge permits, and introduce penalties for non-compliance. |
| **24.** | Provincial Environmental Protection Agencies (Provincial EPAs) | Regulate and monitor environmental pollutants and antimicrobial residues at the provincial level. |
| **25.** | Capital Development Authority (CDA) | Oversee urban infrastructure and development, including AMR monitoring in water and sanitation systems in Islamabad. |
| **26.** | Local Pharmaceutical Companies | Monitor and control antimicrobial use in manufacturing, manage disposal practices, and collaborate in reducing environmental AMR risks. |
| **27.** | Poultry Farmers | Monitor and manage antimicrobial use in poultry farming, ensuring responsible usage and minimizing AMR risks. |
| **28.** | Poultry Association of Pakistan | Advocate for responsible antimicrobial use in poultry farming, develop guidelines, and support AMR prevention efforts within the sector. |
| **29.** | Academia (Universities and Research Institutions) | Conduct research on AMR, develop monitoring methodologies, and provide technical training for environmental surveillance. |
| **30.** | Higher Education Commission (HEC) and Universities | Support research, methodology development, and capacity building for environmental AMR surveillance. |

## 1.9 Alignment with Pakistan's NAP on AMR (2024–2028)

The scope of the AMR Environmental Surveillance Strategy centres on integrating environmental AMR surveillance into Pakistan’s One Health approach, as detailed in the National Action Plan (NAP) on AMR. A three years phased implementation plan (2024–2028) is proposed (*Table-2*), outlining objective-specific steps, timelines, and clearly defined roles across various sectors, including government agencies, civil society, and the private sector. This strategy aims to address environmental factors contributing to AMR, thereby enhancing the nation’s capacity to tackle this pressing public health challenge.

**Table 2: Implementation Plan for the Environmental Surveillance of Strategy on Antimicrobial Resistance (2025-2027)**

| NAP 2.0 Objectives | NAP Sub-Objectives/ Interventions | Key Actions | Target Audience | Output | Responsible Stakeholders | Timeline |
|---|---|---|---|---|---|---|
| Objective-1: Governance and Coordination | Establishment of AMR governance and financing mechanisms | Establish national and provincial AMR Environmental surveillance centers. | Government agencies, International partners | Centralized data-sharing platforms in the form of LIMS | MoNHSRC, MoWR, PCRWR, NIH | 6–12 months |
| | | Develop centralized coordination portal for collaboration among environmental stakeholders. | Government agencies, International partners | A platform to ensure smooth coordination across sectors for AMR management. | MoNHSRC, MoWR, PCRWR, NIH | 6 months |
| | Coordination and harmonization of activities on AMR | Organize workshops and regular stakeholder meetings. | Ministries, academia, private sector | Environmental stakeholder engagement; Defined roles and responsibilities. | MoNHSRC, NIH, MoCC, PCRWR | Quarterly |
| | Strengthening existing rules and regulations across sectors | Integration of AMR Surveillance in national policies/legislations. | Environmental scientists, health professionals | Revised national policies | PCRWR, NIH, Provincial EPAs | 4–6 months |
| | | Develop standardized guidelines for AMR surveillance in environmental compartments. | Environmental scientists, health professionals | Standardized AMR surveillance protocols to ensure uniformity in environmental monitoring. | PCRWR, NIH, Provincial EPAs | 4–6 months |
| Objective-2: Improving Awareness and Understanding of AMR | Establish and implement AMR awareness and behaviour change strategy | Organize public campaigns on AMR risks and remediation. | General public, communities | Increased awareness of AMR risks linked to environmental contamination. | MoNHSRC, NGOs, PCRWR | Ongoing |
| | | Disseminate educational materials on AMR risks. | General public, farmers, environmentalists, academia, healthcare professionals and staff | Widely distributed educational resources on AMR prevention and environmental impact. | MoNHSRC, NGOs, PCRWR Ministry of Federal Education and Professional Training (MoFEPT) Framer's associations Ministry of Agriculture Ministry of Climate Change Ministry of Health | Ongoing |
| | Build capacity in AMR surveillance | Train environmental laboratories, scientists and health professionals on WHONET, LIMS, and surveillance methodologies. | Environmental and health professionals | Trained workforce skilled in AMR surveillance. | PCRWR, NIH, Provincial Health Departments | 6–12 months |
| | Community-based AMR training | Collaborate with academia, local governments, and NGOs for AMR risk awareness. | Academia, local governments | Community-level awareness on AMR risks and mitigation strategies. | PCRWR, NGOs | Ongoing |
| | | Publications and Reports: Regular publications, including policy briefs, case studies, and thematic | General public, policy makers, international agencies | Regular publications on gender-sensitive AMR practices, | MoNHSRC, PCRWR, WHO | Bi-annually |

| NAP 2.0 Objectives | NAP Sub-Objectives/ Interventions | Key Actions | Target Audience | Output | Responsible Stakeholders | Timeline |
|---|---|---|---|---|---|---|
| | | reports on gender-sensitive AMR practices, will be distributed to national and international audiences, enabling knowledge sharing and supporting evidence-based advocacy. | | facilitating advocacy and knowledge sharing. | | |
| Objective-3: Strengthen Surveillance and Research on AMR | Strengthening AMR coordinating centers and reference laboratories | Deploy molecular tools (qPCR, genome sequencing) to monitor critical AMR hotspots (healthcare effluents, agricultural runoff, etc.). | Environmental agencies, healthcare facilities | Comprehensive AMR data from high-risk environments. | PCRWR, NIH, Provincial EPAs | 6–24 months |
| | Integrated AMR surveillance | Centralize surveillance data through PCRWR's National Water Quality Portal. | Government agencies, decision-makers | Centralized AMR data repository for actionable insights. | PCRWR, NIH | 12 months |
| | Genomic surveillance and HAIs surveillance | Investigate AMR transmission pathways; Pilot interventions like bioremediation. | Academia, research institutions | Evidence-based interventions for AMR mitigation. | PCRWR, NIH, WHO | Ongoing |
| | Ensuring quality through SOPs and EQA systems in the health sector | Standardize laboratory methods and conduct external quality assurance (EQA). | Laboratories, health professionals | Reliable and validated AMR data for informed decision-making. | NIH, Provincial Health Departments | 12–18 months |
| Objective-4: Reduce Infections Through IPC Measures | Create a formal structure for IPC policies and strategies for environmental sector | Monitor antimicrobial residues and resistance genes in drinking water and wastewater. | Water utilities, local governments | Comprehensive AMR monitoring in water systems. | PCRWR, WASA, MoWR | 6–18 months |
| | Availability of trained human resources | Develop and enforce IPC protocols for healthcare effluents and wastewater systems. | Healthcare facilities | Improved waste management in healthcare settings, reducing AMR risks. | MoNHSRC, NIH, Provincial Health Departments | 6–12 months |
| | Enable conducive IPC environments | Support development of wastewater treatment plants with AMR mitigation technologies. | Municipal authorities, local governments, international development agencies | Operational wastewater treatment plants targeting AMR hotspots. | MoWR, PCRWR, WASA | 12–24 months |
| | Strengthen animal health and agricultural IPC practices | Implement farm biosafety guidelines; Train agricultural workers on AMR prevention. | Farmers, veterinarians | Reduced AMR risks in agricultural settings. | MoNFSR, Livestock Departments | 12–24 months |
| | Farmers Training Program on Safe Use of Antibiotics | Launch a training program on the safe use of antibiotics in agriculture, including environmental impacts. | Farmers, Framers associations, agricultural workers | Improved safe antibiotic use practices to prevent AMR in agriculture. | MoNFSR, MoA, Provincial Departments | 6–12 months |
| | Community Engagement | Semi-annual community engagement sessions to discuss findings with local leaders and community representatives, ensuring grassroots-level awareness of MEL findings and gender-sensitive AMR practices. | Local community leaders, grassroots organizations | Enhanced community engagement, ensuring localized understanding and ownership of AMR practices. | MoNHSRC, Local Governments, NGOs | Semi-annually |
| Objective-5: Optimize | Strengthening rules on | Develop policies for antimicrobial waste | Healthcare facilities, | Comprehensive policies regulating | DRAP, MoNFSR, MoNHSRC | 12 months |

| NAP 2.0 Objectives | NAP Sub-Objectives/ Interventions | Key Actions | Target Audience | Output | Responsible Stakeholders | Timeline |
|---|---|---|---|---|---|---|
| Antimicrobial Use | manufacturing and use of antimicrobials | management in healthcare, agriculture, and pharmaceutical sectors. | agriculture, industries | antimicrobial waste discharge, minimizing environmental contamination. | Ministry of Health, Provincial Health Departments, | |
| | Measuring antimicrobial use | Integrate environmental considerations into antimicrobial stewardship programs. | Healthcare professionals, veterinarians | Rationalized antimicrobial usage across sectors, minimizing excess or inappropriate use. | MoNHSRC, NIH, DRAP, Ministry of Health, Provincial Health Departments | Ongoing |
| | Implementation of antimicrobial stewardship programs | Establish frameworks for tracking antimicrobial residues in environmental matrices. | Environmental agencies, policymakers | Regular monitoring of antimicrobial residues in the environment to identify and manage hotspots. | PCRWR, Provincial EPAs | 12–18 months |

The above plan aligns directly with the core objectives of the NAP 2.0, facilitating governance, strengthening AMR surveillance, improving public awareness, and optimizing antimicrobial use. Through collaboration between multiple stakeholders, including Ministries of National Health Services, Water Resources, and Federal Health Agencies, the strategy will establish specialized surveillance centers, promote stakeholder engagement, create educational campaigns, and implement effective waste management protocols. Additionally, the plan emphasizes the use of advanced technologies, such as genomic surveillance and molecular tools, to monitor AMR hotspots in high-risk environments.

This approach ensures a multi-sectoral, coordinated response to AMR, addressing not only healthcare settings but also agriculture, the environment, and water systems. The phased timeline for actions supports continuous improvement, while outputs and responsibilities are clearly designated to ensure a collaborative and effective implementation of AMR prevention and control measures in Pakistan. A detailed description of the different components of this plan (*Table-2*) is provided in the subsequent sections of this strategy.

## 1.10 Coordination and Data Sharing Framework for AMR Surveillance in Environment

The **Coordination and Data Sharing Mechanism** for **AMR Environmental Surveillance** in Pakistan is proposed to be a framework that outlines how stakeholders collaborate, communicate, and exchange information to efficiently monitor, assess, and address antimicrobial resistance (AMR) in the environment. This mechanism would ensure that all relevant parties are engaged, data is shared across various sectors, and the implementation of the AMR surveillance strategy is streamlined and effective. In this context, key components of the coordination and data sharing mechanism on AMR environmental surveillance are given as following in *Table-3*. This table provides a clear overview of the key components, their assigned lead and supportive roles, and detailed activities for the effective implementation of the AMR Environmental Surveillance strategy in Pakistan.

**Table 3: Key Components of the Coordination and Data Sharing Mechanism on AMR Environmental Surveillance:**

| Component | Assigned Lead Role & Supportive Role | Activity Details | Timeline after approval of strategy |
|---|---|---|---|
| **Development and placement of Centralized Coordination Platform** | Lead: MoNHSRC | Establish a central body for AMR environmental surveillance coordination. Monitor inter-ministerial and inter-agency collaboration and ensure effective decision-making. | 6 months |
| **Stakeholder Engagement** | Lead: MoNHSRC<br>Supportive roles: PCRWR | Organize regular meetings and workshops with stakeholders and clarify roles and responsibilities as listed in *Table-1*. | quarterly |
| **Standardized Data Collection and Reporting** | Lead: PCRWR<br>Supportive role: DAI Pakistan | Develop standardized data collection protocols for AMR monitoring in environmental compartments (water & soil). Ensure consistency in data reporting across the country. | 4 months |
| **Data Sharing Systems** | Lead: PCRWR<br>Support: MoNHSRC, Provincial PHED, WASAs, EPAs. | Expand and integrate PCRWR's LIMS and National Water Quality Portal into a centralized AMR Data Management Platform. Facilitate real-time data sharing among stakeholders. | 12 months |
| **Communication and Reporting** | Lead: MoNHSRC<br>Support: Provincial Agencies, Academia | Establish formal reporting mechanisms for AMR trends. Share key findings with stakeholders, including regular updates and transparency in reporting. | 6 months |
| **Collaborative Research and Capacity Building** | Lead: Academia<br>Support: WHO, DAI, FAO, Development Partners | Encourage collaborative research initiatives and capacity-building programs for stakeholders on AMR surveillance techniques and data analysis. | 12-36 months |
| **Legal and Regulatory Framework** | Lead: MoNHSRC, MoCC | Develop legal and regulatory frameworks to ensure proper data collection, sharing, and usage for AMR surveillance. Ensure compliance with national and international standards. | 12-36 months |
| **International Collaboration** | Lead: MoPDSI | Facilitate international collaboration with global | 12-36 months |

| Component | Assigned Lead Role & Supportive Role | Activity Details | Timeline after approval of strategy |
|---|---|---|---|
| | | organizations (WHO, UNEP, etc.) for technical and financial support. Align Pakistan's system with global AMR surveillance standards. | |
| **Feedback and Continuous Improvement** | Lead: MoNHSRC | Establish a feedback mechanism for stakeholders to suggest improvements. Conduct regular reviews and assessments of the coordination and data-sharing system. | 12 months |

## 1.11 Expected outcomes

The implementation plan for the Environmental Surveillance Strategy on Antimicrobial Resistance (2025-2027), as outlined in *Table-2*, is designed to achieve several critical outcomes that will significantly enhance the management of AMR. These outcomes include standardized systems, hotspot identification, and targeted interventions to effectively manage antimicrobial resistance (AMR) as depicted in *Figure-3*:

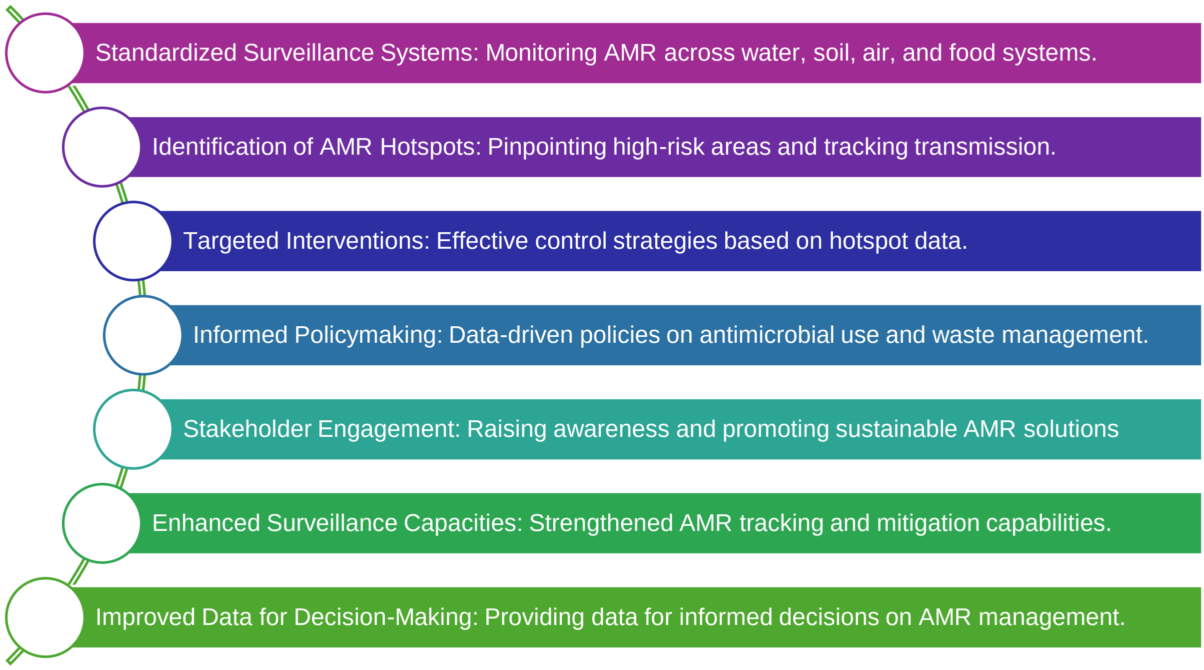


*Figure 3: Expected outcomes of implementation of Strategy for AMR surveillance in the environment*

By focusing on these outcomes, the plan seeks to establish a robust framework for environmental AMR surveillance, contributing to the broader goals of the National Action Plan on AMR and ensuring a coordinated, effective response to this global health challenge.

# 2 Approaches to Environmental AMR Surveillance

Laboratory based AMR surveillance is required for national AMR action plan in the monitoring of emergency and spread of antimicrobial resistance. The generated data will be essential to inform and direct policy on AMR. The goal of this AMR surveillance Framework is to enable standardized, comparable and validated data on AMR to be collected, analysed and shared, in order to inform decision-making, drive local and national action and provide the evidence base for action and advocacy. The AMR surveillance framework for the environment covers samples from waste at point sources (e.g., farms, factories, and community and healthcare settings) as well as locations that act as sinks for waste from point sources, such as river and lakes. The key elements for carrying out antimicrobial resistance surveillance from environment are required to be determined. The laboratory and human resource capacity for such surveillance need to be assessed as well. Surveillance will be phased and progressive.

## 2.1 Methodological Approaches

### 2.1.1 Pakistan's OH Approach in AMR Surveillance Strategy in Environment

Pakistan's AMR Environmental Strategy adopts the One Health concept, acknowledging the interdependence of human, animal, and ecosystem health. This collective approach is essential for effective environmental surveillance of AMR, guided by established frameworks such as the WHO Tricycle protocol (World Health Organization, 2021). Surveillance programs should be tailored to align with environmental risk assessment methodologies while addressing region-specific agricultural, ecological, veterinary, and public health objectives, recognizing that despite varying contexts, the fundamental drivers for AMR surveillance remain globally consistent.

A coordinated, harmonized AMR surveillance strategy will enhance data sharing, foster collaboration, and amplify Pakistan's ability to combat AMR on a wider scale, in line with global initiatives like the WHO Tricycle ESBL project, which integrates environmental surveillance alongside human and animal health. This program uses ESBL-producing *E. coli* as the primary surveillance indicator, utilizing a simple culture-based method similar to those employed in studying third-generation cephalosporin (3GC) resistance in both the human gut microbiome and aquatic environments. This approach has notable advantages: *E. coli* serves as a reliable indicator of faecal contamination and health risks, making it an essential tool in AMR surveillance. Moreover, *E. coli* strains can be serious human pathogens, reinforcing its relevance beyond being a mere proxy or marker. Unlike molecular, culture-independent methods, the Tricycle protocol assesses bacterial viability, a feature valued by many stakeholders. Although the Tricycle method is highly effective in tracking the "circulation of AMR in the environment" as outlined in the WHO action plan (The World Health Organization, 2015) and is used as a mechanism to foster inter-sectoral collaboration, it is not designed to assess the broader impact of human activity on AMR within the environmental microbiome. The methodology it offers could serve as a starting point guidance document for the implementation of AMR surveillance in the environment.

## 2.2 Choice of Surveillance Targets

The choice of surveillance targets for Antimicrobial Resistance (AMR) should be strategically designed to align with the overarching objectives of environmental surveillance programs. The

goal is to focus on critical areas where AMR emerges, persists, and spreads. These targets include Antibiotic-Resistant Bacteria (ARB), Antibiotic Resistance Genes (ARGs), Mobile Genetic Elements (MGEs), and environmental reservoirs, which are crucial in the transmission of AMR as depicted in *Figure-4*. Additionally, surveillance should cover high-risk pathways of resistance spread, antimicrobial use (AMU) patterns, and indicator organisms that signal broader resistance trends. Temporal and spatial patterns are also essential to understand the dynamics of AMR over time and across different geographic areas. The surveillance should further emphasize human-animal-environment interfaces, as these are the hotspots for cross-species and environmental transmission of AMR.


*Figure 4: Choice of surveillance targets for AMR*

Considering above depicted targets, the Environmental Surveillance Strategy specifically proposes monitoring both Gram-positive and Gram-negative bacteria, chosen based on their relevance to human health, persistence in environmental reservoirs, and role in AMR transmission. This targeted approach ensures that critical points in the AMR cycle are systematically monitored to mitigate the risks associated with resistance. The selection of target bacteria is guided by three key criteria.

### 2.2.1 WHO Bacterial Priority Pathogens List (BPPL)

The 2024 WHO BPPL prioritizes 24 antibiotic-resistant pathogens across 15 families, categorized into critical, high, and medium priority groups (World Health Organization, 2024). These serve as global benchmarks for addressing AMR through research, development, and public health interventions. Selection is tailored to organisms of public health significance, their prevalence in Pakistan's environment, and their feasibility for detection.

### 2.2.2 Relevance to Waterborne Outbreaks in Pakistan

Bacteria linked to outbreaks such as typhoid, cholera, and gastroenteritis is prioritized, aligning national surveillance efforts with local public health needs. These pathogens, already included in the WHO BPPL, further validate their inclusion.

### 2.2.3 WHO Global Tricycle Surveillance Protocol

The protocol emphasizes extended-spectrum beta-lactamase-producing Escherichia coli (ESBL-Ec) as an entry point for AMR monitoring, supporting capacity-building for integrated national surveillance systems (World Health Organization, 2021). Moreover, monitoring resistance in Escherichia coli is also a key component of the WHO Global Antimicrobial Resistance Surveillance System (GLASS) main module and is recommended in the WHO/FAO Codex Alimentarius guidelines for foodborne AMR monitoring. This highlights its importance as a universal indicator for antimicrobial resistance surveillance across diverse environments and sectors.

Following above guidelines and considering reported outbreaks in Pakistan as per sector-specific causative agents, Surveillance targets are considered to design a surveillance framework tabulated as *Tables 4 and 5*. This comprehensive framework for initiating AMR environmental surveillance in Pakistan serves as a critical roadmap for systematically addressing antimicrobial resistance within the environment. Following the outlined guidelines and considering the reported outbreaks in Pakistan related to sector-specific causative agents, the surveillance targets have been carefully considered to design an effective surveillance framework, as detailed in *Tables 4 and 5*. By providing a phased approach, the framework ensures a gradual yet robust development of surveillance capabilities, tailored to the specific needs and challenges of the region.

The phased implementation spans distinct timeframes—short-term (0-3 years), medium-term (4-5 years), and long-term (beyond 5 years)—allowing for a step-by-step build-up of infrastructure, expertise, and data management systems. Each phase is strategically designed to focus on key aspects of surveillance, including the identification and sampling of diverse environmental sites, the selection of bacteria and antibiotics for testing, the establishment of surveillance sites across federal and provincial levels, and the integration of advanced data management systems. This comprehensive framework addresses waste monitoring from point sources (such as farms, factories, and healthcare facilities) and environmental sinks like rivers, lakes, and groundwater.

The surveillance targets include bacteria, antibiotics, resistance genes, antibiotics residues, sampling strategies, laboratory support, and training requirements, ensuring a robust foundation for long term surveillance. To avoid challenges associated with result variability and ensure that AMR data are broadly comparable, the framework emphasizes the need for standardized and harmonized laboratory methods, along with the quantitative reporting of AMR data.

Given Pakistan’s resource and capacity challenges, the surveillance approach will be phased over five years, with clear progression through each phase. Institutions like PCRWR, Federal and provincial Environmental Protection Agencies (EPAs), provincial public health laboratories, and laboratories of Water and Sanitation Agencies will lead these efforts, supported by key stakeholders such as the National Institute of Health (NIH), Ministry of National Food Security and Research (MoNFSR), Provincial Health Departments, and DAI Pakistan for capacity building and resource mobilization. This phased approach ensures a consistent and coordinated development of AMR environmental surveillance, strengthening Pakistan’s ability to effectively monitor and combat antimicrobial resistance.

**Table 4: Framework for surveillance of Antibiotic Resistance in Environment**

<table>
<tr><th>Surveillance Targets</th><th>Phase I (0–3 years)</th><th>Phase 2 (4–5 years)</th><th>Phase 3 (> 5 years)</th></tr>
<tr><td>Sites and types of samples (Outflows/ Effluents Sampling)</td><td colspan="3">1. Healthcare settings (human and veterinary): Sewage and effluent.<br>2. Farms (poultry, cattle, pig, fish): Effluent, farm litter/manure, drinking water (for animals), and pond water/sediment (for fish farms).<br>3. Crop farms: Soils, including those where animal farm manure is applied.<br>4. Factory (feed mills, slaughterhouses, processing plants, pharmaceutical. units, and sewage treatment plants (STPs) and effluent treatment plants (ETPs).<br>5. Community settings (STPs and drinking water treatment plants): Effluent (inlet, mid-point, outlet) and drinking water.<br>6. Others (open wells, rivers, lakes, drug disposal sites): Groundwater, river/lakes, surface water, river sediments and soil.</td></tr>
<tr><td>Bacteria isolated from Outflows/ Effluents for AST</td><td>Across all sectors<br>Escherichia coli<br>Enterococcus spp.<br><br>Human-health sector<br>Klebsiella pneumoniae<br><br>Food-animal sector<br>Salmonella spp.<br>Escherichia coli<br><br>Crop sector<br>Aspergillus spp. (fungus)</td><td>Across all sectors<br>Escherichia coli<br>Enterococcus spp.<br><br>Human-health sector<br>Klebsiella pneumoniae<br><br>Food-animal sector<br>Salmonella spp.<br>Escherichia coli<br><br>Crop sector<br>Aspergillus spp. (fungus)<br><br>Aquaculture sector<br>Aeromonas spp.</td><td>Across all sectors<br>Escherichia coli<br>Enterococcus spp.<br><br>Human-health sector<br>Klebsiella pneumoniae<br>Staphylococcus aureus<br><br>Food-animal sector<br>Salmonella spp.<br>Escherichia coli<br>Campylobacter spp.<br><br>Crop sector<br>Aspergillus spp. (fungus)<br>Penicillum spp. (fungus)<br>Fusarium spp. (fungus)<br><br>Aquaculture sector<br>Aeromonas spp.<br>Vibrio spp.</td></tr>
<tr><td>Antimicrobial panel for AST</td><td colspan="3">Escherichia coli: Amoxicillin, cefotaxime, ciprofloxacin, colistin, imipenem, tetracycline and trimethoprim/sulfamethoxazole<br>Enterococcus spp.: Ampicillin, chloramphenicol, ciprofloxacin, erythromycin, levofloxacin, nitrofurantoin, penicillin, tetracycline and vancomycin<br>For sector-specific bacteria: To be based on antibiotics used, resistance trends in humans/animals/ aquaculture/crops and WHO categorization of Critically Important Antimicrobials (CIAs).</td></tr>
<tr><td>Genetic markers (Metagenomic studies and whole genome sequencing (on a subset of isolates) could be considered in long term when capacity allows)</td><td></td><td>ESBL genes (blaCTX-M, blaSHV, blaTEM, etc.)</td><td>ESBL genes (blaCTX-M, blaSHV, blaTEM, etc.)</td></tr>
<tr><td>Federal & Provincial sites</td><td colspan="3">a) Biggest hospitals* one each in federal and provinces (Hospital Wastewater)<br>b) Crop farms one each in federal and provinces<br>c) Poultry farms one each in federal and provinces<br>d) Cattle farm one each in federal and provinces<br>e) Fish farm each one of these in federal and provinces</td></tr>
</table>

| Surveillance Targets | Phase I (0–3 years) | Phase 2 (4–5 years) | Phase 3 (> 5 years) |
|---|---|---|---|
| | f) **Factories** (feed mills, slaughter houses, pharmaceutical industries and their ETPs): Based on concentration of establishments in a region/province or volume of waste generated<br>g) **Sewage Treatment Plants (STPs)**: At least one in federal and all provinces<br>h) **Rivers**: All main rivers in federal and provinces (upstream, mid-stream & downstream)<br>i) **Drains:** All main rivers in federal and provinces<br>j) **Drinking water** point of use (i.e. households), or at the point of production (drinking water production sites) | | |
| Sampling frequency | Bi-annual | Bi-annual | Bi-annual |
| AST interpretation method | Relevant and most recent versions of the CLSI guidance documents (M100, M45 or Vet01) (CLSI, 2022) | Testing Relevant and most recent versions of the CLSI guidance documents (M100, M45 or Vet01) | Relevant and most recent versions of the CLSI guidance documents (M100, M45 or Vet01) |
| Number of laboratories/ networks | • Existing network of laboratories in environmental/public health sector.<br>• Environmental/Public Health labs to be initially supported for laboratory capacity and resources, till necessary capacity for surveillance of AMR in environment are developed. | | |
| Data management** | LIMS, Centralized One Health AMR Dashboard, Global Antimicrobial Resistance Surveillance System (GLASS) | LIMS, Centralized One Health AMR Dashboard, Global Antimicrobial Resistance Surveillance System (GLASS), WHONET software | LIMS, Centralized One Health AMR Dashboard, Global Antimicrobial Resistance Surveillance System (GLASS), WHONET software |
| Training required | Training on AST, GLASS, WHONET, UNEP's Strategic Framework on AMR | Training on AST, WHONET | Training on AST, WHONET |
| Performance Indicators | • Proportion (%) of Institutions incorporated the AMR surveillance into their work plans.<br>• Proportion (%) of targeted resistant pathogens profiles established annually.<br>• Proportion (%) of surveillance sites covered in AMR surveillance annually.<br>• Proportion (%) of surveillance reports generated and shared quarterly, bi-annually and annually.<br>• Number of labs participating in ILC and EQA<br>• Number of policy documents developed<br>• Proportion (%) of early warning notifications resulted from emergence of novel resistant pathogen | | |

* Hospitals being sampled for human-health AMR surveillance could be considered.
** UNEP's Strategic Framework for Collaboration on Antimicrobial Resistance.

## 2.3 Antibiotic Residues in Environment

The framework for surveillance of antibiotic residues in the environment is designed in three phases, each building upon the progress made in the previous one (Table 5). This framework aims to establish a routine and systematic monitoring system, ensuring that antibiotic residues are closely tracked across various environmental sectors. The system is pivotal in addressing the growing concerns of antibiotic contamination, which can contribute to the development and spread of antimicrobial resistance (AMR).

**In Phase I (0-3 years),** the surveillance will focus on critical outflows/effluents from healthcare settings (human and veterinary), including sewage and effluent sources, as well as pharmaceutical units like feed mills and pharmaceutical manufacturing facilities. Antibiotics for monitoring will be selected based on a careful evaluation of usage patterns in human health, veterinary practices, and agriculture, considering resistance trends and the WHO's categorization of Critically Important Antimicrobials (CIAs). Priority will be given to those antibiotics known to contribute significantly to resistance. Water and wastewater samples will be collected bi-annually from these outflows and effluents, with a primary goal to quantify antibiotic residues. Initial analysis will be conducted by the National Water Quality Laboratory of Pakistan Council of Research in Water Resources, supported by stakeholders such as National Institute of Health (NIH), National Reference Laboratory for Food Safety, Pakistan Agricultural Research Council (PARC), and Punjab Agricultural Research Board (PARB), Food and Drug Control Laboratory (FDCL), and Drug Regulatory Authority of Pakistan (DRAP) and focus will be placed on training laboratory staff in sample management, preparation, and the proper use of testing methods to ensure reliable and consistent results.

**In Phase 2 (4-5 years),** the surveillance scope will expand to include agricultural settings, specifically targeting farm effluents from poultry, cattle, and fish farms, where antibiotics are heavily used. Antibiotic selection will continue to be informed by on-going usage patterns and emerging resistance trends across human health, veterinary, and agricultural sectors. Sampling will remain bi-annual, and high-sensitivity testing methods will be utilized to maintain consistency and accuracy in detecting antibiotic residues in agricultural effluents. The laboratory network will expand to include regional laboratories across the provinces, and training will be enhanced to improve laboratory staff's capacity for more advanced testing techniques.

**In Phase 3 (beyond 5 years),** the surveillance will broaden to include an even wider array of agricultural outflows, continuing the focus on poultry, cattle, and fish farm effluents while maintaining surveillance on healthcare and pharmaceutical outflows. The priority will remain on monitoring the most critical antibiotics, in line with emerging resistance trends and UNEP's Strategic Framework on Antimicrobial Resistance/WHO recommendations. This phase will also focus on identifying new trends in antibiotic usage and potential environmental risks.

**Table 5: Framework for surveillance of Antibiotic Residues in Environment**

| | Phase I (0–3 years) | Phase 2 (4–5 years) | Phase 3 (> 5 years) |
|---|---|---|---|
| Sample sites and types | • Healthcare settings (human and veterinary): Sewage and effluent | • Healthcare settings (human and veterinary): Sewage and effluent | • Healthcare settings (human and veterinary): Sewage and effluent |

| | Phase I (0–3 years) | Phase 2 (4–5 years) | Phase 3 (> 5 years) |
|---|---|---|---|
| | • Factories (Feed mills and pharmaceutical units): Sewage and effluent | • Factories (Feed mills and pharmaceutical units): Sewage and effluent | • Factories (Feed mills and pharmaceutical units): Sewage and effluent<br>• Farms (poultry, cattle, pig and fish): Farm effluent and soil |
| Antibiotics for residue monitoring | To be based on antibiotics used, resistance trends in humans, animals, aquaculture, crops and WHO categorization of Critically Important Antimicrobials (CIAs) | | |
| Provinces | 1. Biggest hospitals* one each in federal and provinces<br>2. BHU one each in federal and provinces<br>3. One veterinary hospital each in three provinces<br>4. Pharmaceutical units two in each province<br>5. Crop Farms, Poultry, Cattle, and Fish Farm each one of these in federal and provinces<br>6. Feed mills two each in federal and every province | | |
| Sampling frequency | Bi-annual | Bi-annual | Bi-annual |
| Analytical method | Mass Spectrometry methods such as Liquid Chromatography-Mass Spectrometry (LC-MS/MS), Gas Chromatography-Mass Spectrometry (GC-MS), and High-Resolution Mass Spectrometry (HRMS) | | |
| Number of laboratories/ networks | Existing network of laboratories in environmental sector such as;<br>• Pakistan Council of Research in Water Resources (PCRWR),<br>• Provincial Environmental Protection Agencies (EPAs),<br>• Pakistan Council of Scientific and Industrial Research (PCSIR) | | |
| Training required | Sample management, samples preparation, testing equipment and test methods | Sample management, samples preparation, testing equipment and test methods | Sample management, samples preparation, testing equipment and test methods |

* Hospitals being sampled for human-health AMR surveillance could be considered

As the surveillance system evolves, advanced analytical methods will be employed, and standardized testing protocols will be implemented to ensure consistency and comparability of data across regions and phases. Laboratory infrastructure will continue to develop and strengthen, supporting a broader, more robust network for environmental monitoring. Comprehensive training programs will be reinforced to address the increasing complexity of the surveillance process, ensuring that all laboratories involved can manage the growing demands of the monitoring effort. Through this phased approach, the surveillance system will remain scalable and adaptable, capable of effectively monitoring antibiotic contamination in environmental outflows and contributing to a more sustainable management of antibiotics in Pakistan's environment.

## 2.4 Environmental Sampling for AMR Surveillance

Temporal and geographic patterns in antimicrobial resistance (AMR) reflect the shifting prevalence and spread of antibiotic-resistant organisms and resistance genes across regions and time, influenced by factors such as healthcare practices, agricultural antibiotic use, environmental contamination, and policy measures. Monitoring these patterns helps identify hotspots and guide control strategies. Sampling locations are selected based on high-risk environments where AMR is likely to thrive, including areas with poor sanitation, high population density, extensive agricultural antibiotic use, and significant wastewater discharge as shown in *Figure-5*.

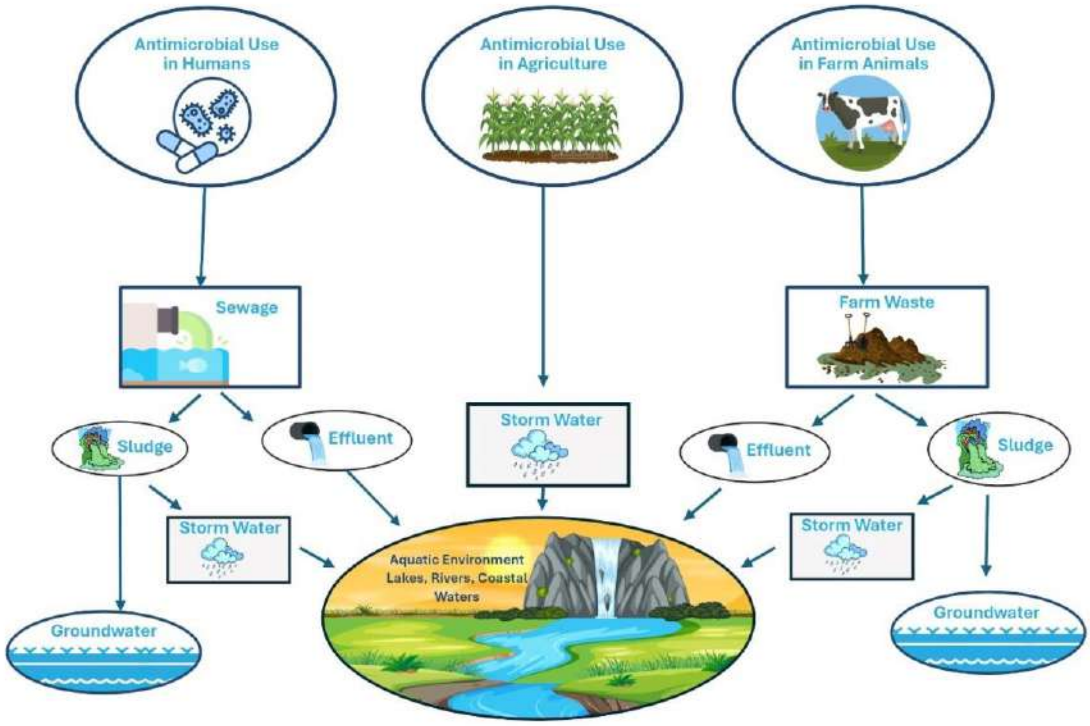


*Figure 5: Choice of environmental surveillance targets for AMR*

Key environmental substrates for AMR surveillance include water, sewage, and soil, which act as critical reservoirs and transmission pathways for antibiotic-resistant bacteria (ARB) and resistance genes (ARGs). Water systems and sewage networks are major conduits for AMR dissemination into both human and animal populations. In this context sampling consideration for AMR environmental surveillance for priority organisms as per *Table-4* is outlined as below in *Table-6*.

**Table 6: Sampling guideline for AMR Environmental Surveillance**

| Sampling Guideline | Details |
|---|---|
| **Sampling Framework** | An abstract transect method may be used to establish the sampling frame and identify a representative subset of the sample population. This approach would ensure systematic coverage of diverse environmental settings (soil, water, and plants) while maintaining spatial consistency. |
| **Sample Size Determination** | The sample size may be determined using randomization to select predetermined numbers. In the absence of sufficient data, anecdotal evidence may guide the selection. |
| **Sampling Frequency** | Active sampling should be conducted quarterly from selected sites to capture temporal variations in AMR prevalence, balancing resource constraints with sufficient data collection. |
| **Site Selection** | Sites should be selected based on risk assessments, focusing on areas impacted by anthropogenic activities such as agricultural runoff, wastewater discharge, and industrial effluents. |
| **Sample Collection** | Approved Standard procedures for sample collection, preservation, transportation and storage should be adhered. |
| **Sample Integrity and Transport** | Samples should be handled, labelled, and transported under controlled conditions to preserve microbial viability and prevent contamination, following standardized protocols. |
| **Sample Submission Form** | All samples submitted to the laboratory for AMR surveillance must be accompanied by a completed sample submission form. The form should include:<br>**Sample Location**: Precise location where the sample was collected.<br>**Premise**: Information about the site from which the sample was taken.<br>**Sampling Date**: The exact date the sample was collected.<br>**Premise Neighboring Activities**: Details on activities occurring around the sampling site that might influence the sample.<br>**Sample Type:** Specify whether the sample is from soil, water, plants, etc. |
| **Data Integration** | Sampling results should be integrated into a centralized database to facilitate trend analysis, geographic comparisons, and integration with other One Health surveillance data streams. |

### 2.4.1 Environmental Laboratory Standards for AMR Surveillance

The minimum Environmental Laboratory Standards tabulated as *Table-7* are basically essential requirements for laboratories to participate in Pakistan's Antimicrobial Resistance (AMR) Environmental Surveillance System. These standards are designed to ensure that participating laboratories are equipped and capable of performing high-quality, reliable testing of environmental samples to monitor antimicrobial resistance (AMR) effectively. Laboratories are required to meet specific technical and organizational criteria, including proficiency in identifying priority organisms and performing Antimicrobial Susceptibility Testing (AST) according to internationally recognized protocols. For example, laboratories must be able to perform AST using disc diffusion, semi-automated, or manual generation of minimum inhibitory concentration (MIC) and gradient diffusion methods following relevant standards for testing such as those of the CLSI.

Furthermore, a robust Quality Control (QC) system would be required to be implemented, in alignment with ISO-17025:2017 standards, to ensure that all results are accurate and consistent. Laboratories must maintain thorough QC data records for internal and external audits, which are crucial for ensuring the reliability of test results. To reinforce accuracy, external quality assurance (EQA) systems must be followed, ensuring that laboratories can perform proficiency tests and achieve acceptable results according to the guidelines of ISO-17025:2017.

Laboratories are also required to have strong data management capabilities, including trained personnel for timely and accurate data collection, analysis, and reporting. This ensures that all data related to AMR surveillance is reliable and accessible for further analysis and decision-making. Laboratories must also ensure that they have the necessary infrastructure, human resources, and microbiological expertise to support the AMR surveillance process effectively. This includes the presence of a Laboratory Information Management System (LIMS) to facilitate data organization and retrieval.

Finally, laboratories are required to maintain an adequate supply of media, reagents, and equipment, with proper adherence to standard operating procedures (SOPs) to ensure the validity of test results. The materials and equipment used must comply with the established standards or SOPs, guaranteeing consistency in testing and minimizing errors. By meeting these minimum requirements, laboratories will be well-equipped to contribute to the AMR surveillance system, ensuring high-quality, reliable data for tracking and managing antimicrobial resistance in the environment.

**Table 7: Minimum Requirements and Specifications for Laboratory Components Supporting AMR Surveillance**

| Components | Minimum Requirements | Details/Specifications |
|---|---|---|
| **Technical Laboratory Skills** | • Identify priority organisms and perform Antimicrobial Susceptibility Testing (AST) as per World Health Organization (WHO) or Clinical and Laboratory Standards Institute (CLSI) standards.<br>• Conduct AST using disc diffusion, semi-automated, or manual Minimum Inhibitory Concentration (MIC) and gradient diffusion methods. | Laboratories must ensure standardization in AST processes and follow internationally recognized protocols. |
| **Quality Control** | • Implement and maintain a Quality Control (QC) system in line with ISO-17025:2017 standards.<br>• Maintain QC data records for internal review and external audits. | A robust QC system must be in place to ensure accuracy and consistency of results, with records available for internal audits or third-party validation as per ISO-17025:2017. |
| **External Quality Assurance (EQA)** | • Ensure laboratories follow ISO-17025:2017 standards for proficiency testing.<br>• Achieve acceptable performance in proficiency | Laboratories should adhere to ISO-17025:2017 guidelines for proficiency testing, ensuring their testing accuracy meets international benchmarks even without an external EQA system. |

| | | |
|---|---|---|
| | tests as per the established guidelines. | |
| **Data Management** | • Commit to collecting and reporting high-quality data within set timelines.<br>• Employ trained staff for data collection, analysis, and reporting.<br>• Dedicate staff time for data input, analysis, and reporting. | Ensure timely and accurate reporting to central systems. Employ qualified personnel in epidemiology, clinical, and laboratory data management. |
| **Site and Laboratory Staff** | • Willingness to participate. Adequate physical infrastructure and equipment.<br>• Adequate human resources. Microbiology expertise among staff.<br>• Information systems. | Laboratories must have sufficient infrastructure, experienced microbiologists, and an effective Laboratory Information Management System (LIMS) to support Antimicrobial Resistance (AMR) surveillance. |
| **Laboratory Supplies, Reagents and Equipment** | Media, reagents, and equipment operation should comply with test standards and SOPs. | Media and reagents must follow test standards. If selective media is not specified, use manufacturer instructions. Equipment operation should follow SOPs with test conditions based on standards or SOPs. Ensure availability of necessary supplies. |

## 2.5 Test Methods for Antimicrobial Resistant Bacteria (ARB)

ARB refers to the process of identifying and analysing bacteria that have developed resistance to one or more antimicrobial agents. ARB testing plays a crucial role in detecting, monitoring, and managing antimicrobial resistance (AMR) in environmental context. Laboratory testing standards for antibiotic resistance monitoring in the environment under this strategy would encompass following four categories of methods:

| Culture-dependent methods | Culture-independent methods |
|---|---|
| Culture-dependent methods isolate and grow microorganisms from environmental samples on selective media to detect and characterize antibiotic-resistant bacteria and resistance genes. | Culture-independent methods analyze DNA/RNA extracted from environmental samples to detect antibiotic-resistant genes and microbial communities without the need for culturing. |
| Phenotypic characterisation methods | Metagenomic (and metatranscriptomic) approaches |
| Phenotypic characterisation methods assess the observable traits of microorganisms, such as antibiotic resistance patterns, through techniques like disk diffusion and MIC testing. | Metagenomic and metatranscriptomic approaches analyse total DNA and RNA from environmental samples to explore microbial diversity, identify resistance genes, and understand their expression in various conditions. |

## 2.6 Culture-dependent Methods for AMR Surveillance in Environment

### 2.6.1 Detection of AMR Bacteria

International standard methods are suggested to be used in accordance with the surveillance targets. These may include standard protocols for ARB and AST as published by the WHO, APHA, USPA, ISO etc. These methods include culture-based detection and enumeration of the target organisms, biochemical and serological characterizations of the target bacteria. Some of these are for instance as below:

i. WHO integrated global surveillance on ESBL-producing E. coli using a "One Health" Approach: implementation and opportunities. Geneva: World Health Organization; 2021. License: CC BY-NC-SA 3.0 IGO.

ii. American Public Health Association (APHA) Methods for Pathogenic Bacteria Detection (2023)

   - ***Salmonella* Typhi**: Method 9260, Standard Methods for the Examination of Water and Wastewater.
   - ***Vibrio cholerae***: Method 9260, Standard Methods for the Examination of Water and Wastewater.
   - Enterococcus spp:
   - Method 9230, Standard Methods for the Examination of Water and Wastewater.
   - ***Klebsiella pneumoniae***: Method 9222, Standard Methods for the Examination of Water and Wastewater.
   - ***Aeromonas* spp**: Method 9262, Standard Methods for the Examination of Water and Wastewater.
   - ***Campylobacter* spp**: Method 9264, Standard Methods for the Examination of Water and Wastewater.
   - ***Aspergillus* spp** (Fungi): Method 9610, Standard Methods for the Examination of Water and Wastewater.

iii. ISO methods for detection of above bacteria as listed at https://www.iso.org/standards.html

### 2.6.2 Confirmation of Isolates

Confirmation should be performed using advanced and recommended techniques, including:

- Serotyping (e.g., for *Salmonella spp*).
- Polymerase Chain Reaction (PCR) for *E. coli* and *Staphylococcus aureus.*
- ESBL-specific Chromogenic media and biochemical tests.
- For certain species, pathogen identification at the type level (e.g., serotypes) may be necessary.

These methods ensure consistency and reliability in detecting and characterizing AMR bacteria, contributing to effective surveillance and management strategies.

### 2.6.3 Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing for priority pathogens shall be carried out in line with Clinical Laboratory Standards Institute (CLSI) guidelines, using the disc diffusion method. The laboratories shall document whether isolates are susceptible (S), intermediate (I) or resistant (R) according to clinical breakpoints defined by CLSI together with the zone sizes (mm) to allow for retrospective adjustment if new breakpoints are set. As the laboratory performance becomes acceptable, minimum inhibitory concentrations (MICs) may be determined by broth dilution (manual or automated) or gradient diffusion tests such as E-Tests. MIC values shall be recorded.

AST will be performed for the antimicrobial groups mostly used in Pakistan and the UNEP/WHO recommended panel. For example the classes belonging to; fluoroquinolones, carbapenems, tetracyclines, penicillins, sulfonamides, 3$^{rd}$ and 4$^{th}$ generation cephalosporins, aminoglycosides, and macrolides. Quantitative results (disc diffusion, zone diameters or minimum inhibitory concentration values) will be performed in accordance to CLSI standards.

i. Guideline, C.L.S.I., 2016. M45; Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria.

ii. Clinical and Laboratory Standards Institute (CLSI). (2022). M100: Performance Standards for Antimicrobial Susceptibility Testing. 32$^{nd}$ Edition. Wayne, PA: CLSI.

### 2.6.4 Use of VITEK 2 in AMR Surveillance in Environment

The VITEK 2 system, while not a standard method defined by international standard-setting bodies such as ISO or CLSI, is a widely recognized and validated automated tool extensively used in AMR surveillance and clinical microbiology. It provides rapid and reliable identification of bacteria and (AST, making it an invaluable resource for analyzing AMR in various environmental samples. The system operates within standardized frameworks, adhering to regulatory approvals like CE marking, and follows AST guidelines established by CLSI or EUCAST (some antibiotics). AMR Environmental strategy supports the integration of VITEK 2 into environmental laboratories workflows through standard operating procedures (SOPs) that align with accreditation requirements, such as ISO 17025:2017 for testing laboratories. Currently, Laboratories of PCRWR and National Institute of Health have the facility of VITEK 2 which would be utilized for AMR environmental surveillance.

## 2.7 Culture-independent Methods for AMR Surveillance in Environment

Culture-independent methods (CIMs) are pivotal in AMR environmental surveillance, enabling the analysis of genetic material and other molecular components directly from environmental samples such as water, soil, wastewater, sediments, and air. By passing the need for culturing microorganisms, which can miss uncultivable or slow-growing microbes, these methods provide a more comprehensive understanding of the environmental resistome. However, they come with challenges, including high resource demands in terms of manpower, equipment, operational costs, consumables, and supplies. Despite these challenges, CIMs provide critical insights into the prevalence and spread of antimicrobial resistance in ecosystems, but also face issues such as complex bioinformatics analysis and the need for robust databases to accurately identify antimicrobial resistance genes (ARGs).

Key culture-independent methods include Quantitative PCR (qPCR) and Whole Genome Surveillance.

**qPCR** is a powerful tool for quantifying specific ARGs and mobile genetic elements, such as integrons and plasmids, in environmental samples. It offers high sensitivity and specificity, making it ideal for tracking the abundance of well-known ARGs, including those conferring resistance to β-lactams, tetracyclines, sulfonamides, and other antibiotics.

**Whole Genome Surveillance** involves sequencing the entire genome of microorganisms directly from environmental or clinical samples, providing a comprehensive analysis of their genetic composition, including AMR genes, virulence factors, and microbial diversity. This method utilizes high-throughput sequencing technologies to analyse genomic DNA, allowing for the detection of both known and novel resistance mechanisms, as well as the tracking of resistance spread through genomic epidemiology. Whole genome surveillance is invaluable for detecting emerging resistance patterns, offering detailed genetic data, and supporting the One Health approach by monitoring AMR across human, animal, and environmental interfaces.

Currently, National Institute of Health has these facilities and is declared as National Reference Laboratory, therefore services of NIH may be utilized for metagenomics analysis of environmental samples.

## 2.8 Quality Assurance (QA)

AMR Environmental Surveillance Labs will establish a Quality Assurance (QA) system for environmental labs involved in Antimicrobial Resistance (AMR) Surveillance in Pakistan, the following QA requirements should be ensured:

- Availability of competent technical personnel for accurate testing and analysis.
- Implementation of a robust Quality Management System (QMS) to maintain consistent quality and compliance with standards.
- Participation in intra- and inter-laboratory comparison testing to validate results and ensure proficiency.
- Regular internal and external quality audits, with corrective actions for non-conformance identified during audits.
- Provision of reference materials for each priority pathogen to minimize user errors and ensure correct identification.
- Inclusion of positive and negative controls in each test runs to ensure accuracy of results.
- Retention of QC data sheets and corrective action summaries for documentation and accountability.
- Periodic review of all QC documentation by the Centralized Laboratory (CVL).
- Supervisory reviews of out-of-range QC results, with documentation of corrective actions for all QC failures.

### 2.8.1 Internal Quality Control (IQC)

To ensure the quality and reliability of results in AMR Environmental Surveillance labs in Pakistan, the following IQC requirements must be met:

- Perform regular IQC testing, with frequency determined by specimen load; each batch of AST should ideally include an IQC.

- Ensure laboratory equipment is regularly calibrated, maintained, and assessed for quality.
- Use reference bacterial strains, in accordance with CLSI guidelines, to monitor the accuracy of test results.
- Ensure control strain results fall within expected ranges; any deviations require troubleshooting and corrective actions by technical personnel.
- Conduct IQC prior to AST on isolates, using control strains sourced from ATCC.
- Onsite evaluation visits and retesting frequency will be determined by the site visit schedule.

### 2.8.2 External Quality Assurance (EQA)

To ensure the quality and reliability of results in AMR Environmental Surveillance labs in Pakistan, the following EQA requirements will be met:

- **Participation in Proficiency Testing (PT)**: Lab will participate in PT, re-testing, and on-site evaluations organized by international and reference labs for AMR surveillance.

- **Timely Reporting**: PT results must be reported according to instructions and submitted within required deadlines to the agency conducting EQA, allowing for feedback and comparisons with other laboratories.

- **Regular EQA and QC Performance**: Lab will conduct EQA assessments at least once a year, and whenever possible, twice per year to assess overall performance and ensure continuous quality.

## 2.9 Isolate Repository

All banked cultures will have a repository at National reference AMR laboratory e.g. NIH Islamabad.

- Surveillance sample collection sites will send AMR priority isolates to the reference laboratory (NIH) monthly.
- Isolates will be stored at -20°C or -80°C during transport and prior to storage, following the standard SOPs.
- Repositories will require to be equipped with ultra-low freezers, a generator, and an electronic specimen tracking system with bar-coding for easy retrieval.
- A freezer manager will oversee the repository, ensuring proper storage and tracking of isolates.
- The repository will support nationally relevant research by housing isolates and sources from the AMR surveillance network.
- Quarterly, 10% of isolates with priority AMR organisms will undergo confirmatory testing.
- National reference AMR laboratory such as NIH will investigate and correct any discrepant results from confirmatory testing.

## 2.10 GIS/Remote Sensing for AMR Hotspots Mapping

Integrating GIS and remote sensing technologies into Pakistan's AMR environmental surveillance strategy will enable the identification and monitoring of antimicrobial resistance (AMR) hotspots with greater precision and efficiency. By utilizing satellite imagery, drones, and geospatial tools, these methods can identify critical sources of AMR pollutants, including pharmaceutical manufacturing facilities, wastewater discharge sites, and agricultural runoff areas. GIS and remote sensing will facilitate real-time monitoring of environmental parameters such as water quality, land use, and industrial activity, offering valuable insights into regions most at risk for AMR contamination. Spatial analysis and risk mapping will allow for the visualization of AMR trends and the identification of high-priority areas for targeted interventions. By incorporating GIS and remote sensing into the AMR surveillance framework and building technical capacity, Pakistan can strengthen its ability to track, analyze, and address AMR across diverse environmental contexts, protecting public health and ecosystems.

## 2.11 Surveillance Data Management and Information Sharing

The data generated by AMR surveillance across the country keeps immense potential to drive policy decisions. With implementation of AMR Environmental Strategy, it would be imperative to have tools to integrate the data generated across the country into a single data repository.

As part of Pakistan's AMR Environmental Surveillance Strategy, it is proposed that all environmental laboratories implement Laboratory Information Management Systems (LIMS) to streamline the collection, management, and analysis of environmental AMR data. Under this plan, AMR data from environmental samples will be directly captured and stored within the LIMS at each environmental laboratory. The process flow will work as follows:

- **Data Capture in LIMS:** Environmental AMR data, including resistance profiles and gene sequencing results, will be inputted into LIMS at the laboratory level. LIMS will act as the central hub for managing all AMR-related data, ensuring data accuracy, consistency, and easy retrieval for further analysis.

- **Data Transfer to Centralized One Health AMR Dashboard:** A quarterly online system in the form of Centralized One Health AMR Dashboard for reporting of surveillance data will be developed. Once AMR data is captured in the Laboratory Information Management System (LIMS); it will be automatically transferred to the Centralized One Health AMR Dashboard. This centralized portal will function as the national repository for all environmental AMR data, integrating information from across the country. The dashboard will support real-time monitoring, comprehensive data analysis, and streamlined reporting, thereby facilitating informed decision-making and effective policy development.

- **Submission to relevant global surveillance initiatives:** Data from the Centralized One Health AMR Dashboard will be formatted according to the requirements of UNEP's Strategic Framework for Collaboration on Antimicrobial Resistance. Accordingly, the dashboard will be equipped with the necessary tools to submit AMR data to relevant global AMR surveillance efforts (led by UNEP, the responsible quadripartite agency for AMR surveillance in the environment) and ensuring Pakistan's alignment with international standards for AMR monitoring.

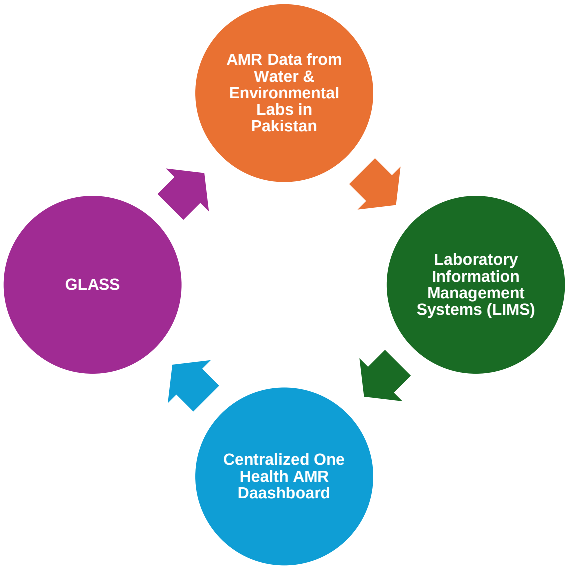


*Figure 6: Plan for Data Management and Information Sharing*

By centralizing environmental data in Centralized OH-AMR Dashboard (Figure 6), this strategy will propose data accessibility, accuracy, and timeliness, enabling more effective interventions at the national and international levels. The data analysis is suggested to be displayed to the user in the form of pie charts, bar charts, stacked bar charts, and tables, demonstrating the comparable and specific isolation percentages and susceptibility variations. Following same, the targets set under this strategy are mainly:

i. Establish a LIMS for real-time data entry, tracking, and reporting across all surveillance sites.
ii. Create a secure, cloud-based centralized OH-AMR Dashboard for storage and sharing of AMR surveillance data.
iii. Standardize data formats for easy aggregation and analysis, and provide visualization tools (e.g., GIS) for trend identification.
iv. Enable seamless data sharing between stakeholders (government, research institutions, international organizations).
v. Integrate national AMR data with appropriate global surveillance platform (AMR from environmental sources) for global reporting and benchmarking.
vi. Analyze AMR trends using the OH-AMR Dashboard and LIMS, and provide decision-makers with evidence-based insights.
vii. Set up automated alerts for emerging resistance patterns to aid timely response.
viii. Generate regular, standardized reports for stakeholders to inform policy and action.
ix. Provide ongoing training for laboratory staff on LIMS usage, data quality control, and appropriate global platform reporting of AMR data from the environment.
x. Regularly assess and upgrade data management systems to incorporate new technologies and user feedback.
xi. Ensure public access to aggregated AMR data through interactive OH-AMR Dashboard while maintaining confidentiality.

## 2.12 Artificial Intelligence and Machine Learning for Predictive Modelling and Trend Analysis

Artificial Intelligence (AI) and Machine Learning (ML) have the potential to significantly enhance the effectiveness of Pakistan's AMR environmental surveillance strategy. By leveraging these advanced technologies, Pakistan can predict AMR trends, model environmental contamination pathways, and develop data-driven mitigation strategies to combat AMR in the environment. The integration of AI/ML into Pakistan's AMR environmental surveillance framework will allow for a more proactive, evidence-based approach, optimizing resource allocation, early detection, and targeted interventions.

- **Predicting AMR Trends:** AI and ML can be used to analyze historical surveillance data to identify trends and predict future patterns in AMR across various regions of Pakistan. By processing data from multiple environmental monitoring sources (e.g., water, soil, effluents), these tools will help forecast the emergence of resistance in certain areas. These predictive insights will enable timely interventions and resource allocation, addressing AMR challenges before they escalate.
- **Modeling Environmental Contamination Pathways:** ML models can simulate and map the pathways through which antibiotic-resistant bacteria are transported and dispersed in Pakistan's environment. By considering factors such as water flow patterns, agricultural practices, and wastewater management, AI/ML can identify key environmental contamination sources. This insight will support more effective monitoring and management of high-risk areas, such as those near agricultural zones, healthcare facilities, and wastewater treatment plants.
- **Identification of Hotspots:** AI and ML will help detect and predict AMR hotspots—areas where resistance is most likely to proliferate. By analyzing data from environmental samples, including wastewater, healthcare facilities, and agricultural runoff, these technologies will pinpoint regions requiring immediate attention. Identifying these hotspots will improve surveillance efforts and guide resource deployment to areas most at risk.
- **Mitigation Strategies:** AI/ML can assist in developing and simulating various strategies for mitigating AMR in the environment. Based on the trends and hotspots identified, these models can recommend evidence-based interventions such as changes in antibiotic use, improvements in wastewater treatment, or better waste management practices. These insights will support policymakers and stakeholders in Pakistan in implementing the most effective measures to reduce AMR risks.

**Real-Time Surveillance and Continuous Learning:** AI and ML systems can continuously update and adapt as new data is collected. Real-time analysis of environmental samples and effluent data can detect sudden increases in resistance levels, providing immediate alerts. This dynamic approach will enable Pakistan to respond rapidly to emerging AMR threats and continuously refine its surveillance strategies.

## 2.13 AI/ML Capacity Building for AMR Trend Prediction in Pakistan

To effectively integrate AI/ML into Pakistan's AMR environmental surveillance strategy, capacity building at various levels will be essential. The following steps outline how this can be achieved:

- **Training for Government Agencies and Laboratories**: Pakistan's Ministry of National Health Services, Regulation & Coordination (MoNHSRC), Ministry of Climate Change and Environmental Coordination (MoCC), Ministry of Water Resources (MoWR) and associated environmental and public health laboratories, such as the labs of Pakistan Environmental Protection Agency (PEPA), Pakistan Council of Research in Water Resources will need specialized hands-on training on data collection, AI/ML model development, and interpretation of predictive results.

    1) **Collaboration with International Experts and Institutions**: Pakistan will engage with international organizations, universities, and research institutions that specialize in AI/ML applications in environmental health and AMR surveillance. Collaborative programs will be developed to build local expertise, with technical support for setting up predictive modeling systems and refining analytical methodologies.
    2) **Developing AI/ML Infrastructure**: Pakistan will need to invest in the necessary infrastructure for AI/ML applications, including computing power, software, and data storage solutions. This infrastructure will support the collection and analysis of large datasets from environmental surveillance and provide the computational capacity needed for machine learning models.
    3) **Integration of AI/ML in Surveillance Networks**: The capacity building strategy will focus on integrating AI/ML into Pakistan's existing AMR surveillance networks, such as those run by PCRWR and other provincial authorities. This integration will enable real-time data collection, analysis, and decision-making, creating a more responsive surveillance system.
    4) **Building a Network of AI/ML Experts**: To sustain the growth of AI/ML capacity in AMR environmental surveillance, Pakistan will develop a network of local AI/ML experts. This network will provide ongoing training, support for AI/ML model refinement, and continuous development of new methodologies to improve surveillance accuracy and effectiveness.
    5) **Data Quality and Standardization**: To ensure the effectiveness of AI/ML tools, Pakistan will prioritize data quality and standardization. Clear protocols will be established for data collection, labeling, and storage to facilitate the accurate training of AI/ML models. National guidelines for data management will also be developed to ensure consistency and reliability across surveillance sites.

In conclusion, incorporating AI/ML into Pakistan's AMR environmental surveillance strategy will enhance the nation's ability to predict, monitor, and mitigate AMR risks. Through comprehensive capacity building, training, and collaboration with international experts, Pakistan will develop the skills and infrastructure required to fully harness the power of AI/ML, enabling a more robust and proactive approach to AMR surveillance.

## 2.14 National Reference Laboratory for AMR Surveillance in Environment in Pakistan

The relevant ministries/governments in Pakistan such as Ministry of Water Resources, Ministry of Climate Change and Environmental Coordination, Provincial Governments will declare the **National Reference Laboratory (NRL) for Environmental AMR Surveillance** in Pakistan. NRL will play a pivotal role in coordinating and supporting AMR environmental

surveillance activities nationwide. The criteria for declaring any AMR Environmental Surveillance Laboratories as NRL includes; **Advanced Testing Facilities, Skilled and Sufficient Manpower, Bio-Repository and Confirmation Services, Data Management and Reporting system. Expected contribution of NRL will include following:**

a) Maintain advanced diagnostic capabilities, including antimicrobial susceptibility testing (AST), supported by skilled professionals and participation in internationally recognized External Quality Assurance (EQA) programs.

b) Manage a centralized biorepository for key organisms/antimicrobial combinations and confirm novel resistance patterns before reporting to authorities.

c) Host and oversee the national AMR environmental surveillance database, ensuring timely data analysis, reporting, and sharing with relevant stakeholders.

d) Conduct and disseminate research on AMR in environmental compartments to inform national and international policies and action plans.

e) Collaborate with the relevant ministries and sentinel laboratories to standardize microbiological results and support efficient operations through technical guidance and procurement assistance.

This comprehensive set of responsibilities will position the NRL as the central hub for environmental AMR surveillance, ensuring alignment with national priorities and global standards.

## 2.15 National AMR Surveillance Technical Working Group

The Technical Working Group (TWG) on AMR Environmental Surveillance is proposed to be constituted. This TWG may entrust to provide technical support during the implementation of AMR Environmental Surveillance in line with Pakistan's National Action Plan (NAP) for AMR. The TWG will consist of experts with technical specializations relevant to environmental AMR surveillance, including professionals in microbiology, epidemiology, laboratory sciences, environmental health, animal health, infection prevention and control, pharmaceuticals, and water and sanitation. The TWG will act as the focal technical body, ensuring alignment with Pakistan's commitments under international frameworks such as the UNEP's Strategic Framework on Antimicrobial Resistance and WHO Global Action Plan on AMR while addressing national priorities for AMR mitigation in the environment. This TWG will also collaborate and interact with the sector specific TWGs on AMR surveillance in the human health, food, agriculture and animal health. Role and responsibilities of the Technical Working Group (TWG) on AMR Environmental Surveillance shall include:

a) Operationalizing the National AMR surveillance strategy in the environment sectors

b) Providing technical input, conducting situational analyses in the environmental sector

c) Preparing annual work plans as per National AMR Action Plan

d) Providing updates on on-going activities and provide technical advice

e) Prepare annual AMR surveillance work plan in the environment

f) Map AMR surveillance activities in the environment sectors

g) Prepare budget for AMR surveillance

h) Prepare consolidated report for AMR surveillance in the environment sector.

The TWG should meet at least quarterly (every 3 months) for review. These meetings would serve as an opportunity to:

1. Review the progress of ongoing activities.
2. Provide technical updates and advice on AMR environmental surveillance.
3. Discuss situational analyses and new findings in the environmental sector.
4. Assess and align with Pakistan's National Action Plan (NAP) for AMR.
5. Review and update the annual work plan and budget.
6. Prepare and finalize the consolidated report on AMR surveillance activities in the environment sector.

This frequency allows for consistent monitoring, course correction, and alignment with national and international AMR commitments, while providing a structured platform for collaboration with sector-specific TWGs in human health, food, agriculture, and animal health.

## 2.16 Global and Regional Collaboration

To effectively manage environmental AMR risks, Pakistan must collaborate with regional and international partners to align with global AMR strategies and share best practices. This collaboration is crucial for leveraging collective expertise and resources in the fight against antimicrobial resistance.

- **Participation in International AMR Forums:** Pakistan should strengthen its engagement in key global AMR forums, such as the World Health Assembly (WHA), the Quadripartite Alliance on AMR (WHO, FAO, WOAH, UNEP), and the Global Health Security Agenda (GHSA). By participating regularly, Pakistan can stay informed on the latest global strategies, scientific advancements, and emerging trends in addressing environmental AMR, ensuring that national efforts are aligned with global progress.

- **Adoption of International Best Practices:** Collaborating with international organizations such as WHO, UNEP, FAO, and WOAH is essential to adopting and implementing best practices for antimicrobial use, waste management, and environmental protection. For instance, Pakistan can benefit from WHO's guidelines on environmental AMR monitoring, and UNEP's recommendations on managing pharmaceutical waste, which can help reduce environmental contamination and improve public health outcomes.

- **Harmonizing National Standards with International Guidelines:** It is vital to align Pakistan's environmental and health regulations with international guidelines, particularly those focused on the environmental impact of AMR. This includes adopting WHO's water safety standards, UNEP's industrial effluent management guidelines, and FAO's antimicrobial use regulations in agriculture. Harmonizing national standards with these guidelines will ensure a consistent approach to AMR management, facilitate better environmental protection, and enhance public health efforts.

## 2.17 Legal Framework and Resource Mobilization for the Strategy Implementation

The successful implementation of the AMR Environmental Surveillance Strategy in Pakistan requires robust managerial frameworks to ensure seamless coordination among key stakeholders. Strengthening the legislative framework is crucial for the effective enforcement of this strategy. The strategy aligns closely with Pakistan's National Action Plan on AMR (2024–2028) and builds on existing policies and legislation that govern sectors such as health, agriculture, food, water, sanitation, and the environment. Key policies and legislation integral to the implementation of this strategy include:

1) National Water Policy (2018)
2) National Climate Change Policy (2012, updated 2021)
3) Climate Change Gender Action Plan (2022)
4) National Adaptation Plan (2023)
5) National Health Policy (2009, under review for updates)
6) National Livestock Policy (2007)
7) National Drinking Water Policy
8) National Sanitation Policy
9) Provincial Water, Drinking Water, and Climate Policies
10) Provincial Water Acts/Ordinances, e.g., Punjab Water Act (2019), KP Water Act (2020)
11) Animal Disease Act (2015)
12) Punjab Animal Health Act (2019)
13) Pakistan Food Safety Act (2010)
14) Punjab Fisheries Ordinance (1961, amended in 2022)
15) Pakistan Plant Quarantine Act (1976)
16) Pakistan Environmental Protection Act (PEPA) 1997

This strategy is further supported by several related plans and frameworks, including:

1. Pakistan Vision 2025, which emphasizes health security and sustainable development.
2. One Health Framework (2022), promoting interdisciplinary collaboration.
3. National Strategic Framework for Livestock Development (2020-2025).
4. Pakistan Agricultural Research Vision 2030.

In addressing AMR environmental surveillance, Pakistan's regulations should be aligned with the National Environmental Quality Standards (NEQS). However, most existing environmental legislations and regulations currently do not address the critical issue of antimicrobial resistance (AMR). To effectively combat AMR, these regulations need to be updated by its custodian ministry/agency to include specific provisions on AMR. This will encompass governing pharmaceutical companies' compliance with antibiotic discharge limits, controlling livestock and agricultural runoff, and regulating aquaculture antibiotic use and wastewater disposal. Monitoring high-risk environments, including sewage treatment plants and livestock farms, is essential. Additionally, updating these regulations to explicitly include AMR will ensure comprehensive oversight and accountability, with effective inter-agency collaboration being vital for robust monitoring and regulatory adherence. Moreover, Pakistan should adopt international best practices in AMR management, foster collaboration with neighboring countries on cross-border environmental impacts, and invest in innovative wastewater treatment technologies to mitigate AMR. Continuous data collection and research will play a pivotal role in refining regulatory strategies.

In alignment with regional and global efforts, this strategy integrates key international frameworks and action plans, such as:

- UNEP’s Strategic Framework on Antimicrobial Resistance
- WHO Global AMR Action Plan (2015)
- FAO Action Plan on AMR (2016-2020)
- OIE Global Strategy on AMR
- WTO Sanitary and Phytosanitary (SPS) Measures
- Commitments under SAARC and ECO for regional cooperation

This comprehensive approach ensures that Pakistan’s AMR Environmental Surveillance Strategy addresses national priorities while aligning with international standards and commitments. The One Health approach underscores the need to integrate human, animal, and environmental health in legislative frameworks, following guidance from organizations like UNEP’s Strategic Framework on Antimicrobial Resistance, World Health Organization (WHO), the Food and Agriculture Organization (FAO) and World Organisation for Animal Health (OIE). Following the same, the strategy resonates with initiatives led by organizations such as WHO, FAO, the World Organisation for Animal Health (WOAH), and the United Nations Environment Programme (UNEP). These organizations collectively advocate for integrating gender perspectives within AMR frameworks to promote equitable, sustainable, and inclusive responses.

## 2.18 Coordination, Capacity Building & Awareness Planning

Enhancing the capacity of environmental stakeholders and laboratories is crucial for strengthening AMR surveillance and ensuring effective risk management. This involves upgrading laboratory infrastructure, improving technical expertise, and providing comprehensive training on advanced AMR detection methodologies. These capacity-building efforts are closely linked with awareness initiatives, as a technically proficient workforce is essential for effectively communicating AMR risks and mitigation strategies to the public. A phased approach will support the incremental development of surveillance systems, enabling robust data collection and analysis to guide informed decision-making. As laboratories and stakeholders build their capacity, they will also contribute to public awareness campaigns, utilizing their expertise to educate communities about the socio-economic and environmental impacts of AMR.

Pakistan's second National Action Plan (NAP 2.0) on AMR (2024–2028) emphasizes a multi-sectoral One Health approach, building upon the foundation laid by the initial NAP (2017–2021). The previous plan identified critical areas for improvement in AMR management. The 2024–2028 NAP advances these efforts, focusing on governance, awareness, and education across sectors. By integrating comprehensive measures, the current strategy ensures that AMR responses are robust and address the complex challenges posed by antimicrobial resistance, particularly in rural and underserved communities. This inclusive approach aims to create a more effective and coordinated response to AMR challenges.

Raising awareness about AMR is vital in Pakistan due to factors such as widespread antibiotic misuse, inadequate sanitation, environmental contamination, and insufficient regulatory measures. These challenges exacerbate the AMR crisis, threatening public health by rendering existing antibiotics ineffective. By integrating capacity-building efforts with public outreach campaigns, stakeholders can effectively promote responsible practices, such as proper antibiotic use and waste management. The AMR Environment Awareness Plan for Pakistan

(2025-2027) as given in *Table-8* outlines how capacity-building initiatives will empower stakeholders to lead impactful public education efforts, thereby enhancing both surveillance capabilities and community engagement.

**Table 8: Plan for raising AMR Environment Awareness through Capacity Building and Public Outreach Campaigns (2025-2027)**

| Sr # | Activity | Key Responsible Organizations | Mode of Communication | Supporting Organizations | Frequency |
|---|---|---|---|---|---|
| 1. | Strengthen collaboration between ministries for a unified AMR response | MoNHSRC, MoNFSR, MoCC, MoIP | Meetings, reports, workshops | Drug Regulatory Authority of Pakistan (DRAP), Provincial Health Departments | Regular |
| 2. | Establishment of a National One Health Coordination Committee | MoNHSRC, MoNFSR, MoCC, MoIP | Committee meetings, reports | | Once, with follow-ups |
| 3. | Regular Inter-Ministerial Meetings | MoNHSRC, MoNFSR, MoCC, MoIP | Meetings, teleconferences | DRAP, Provincial Health Departments, Agricultural Authorities | Quarterly |
| 4. | Joint Action Plans for environmental AMR mitigation | MoNHSRC, MoNFSR, MoCC, MoIP | Joint meetings, action plan documents | EPA, MNFSR, Ministry of Climate Change | Annually |
| 5. | Strengthen and integrate cross-sectoral AMR surveillance systems | MoNHSRC, MoNFSR, MoCC, MoIP | Data sharing platforms, reports | EPA, Agricultural Authorities, Hospitals, Pharmaceutica l Plants | On-going/ Continuous |
| 6. | Develop multilingual media content on AMR causes and impacts, including improper antibiotic disposal in the environment. | MoNHSRC, MoCC, MoIB | Radio, TV, social media, print media | WHO, NGOs | Quarterly |
| 7. | Organize community workshops and public forums on AMR environmental impact. | MoNHSRC, PCRWR, Provincial EPAs Local Government | In-person workshops, community meetings | WHO, Local NGOs | Bi-annual |
| 8. | Conduct training programs for healthcare workers, farmers, and wastewater treatment plant staff. | MoNHSRC, MoNFSR | Training sessions, seminars, workshops | WHO, FAO, Provincial Agriculture Departments | Annual |

| Sr # | Activity | Key Responsible Organizations | Mode of Communication | Supporting Organizations | Frequency |
|---|---|---|---|---|---|
| 9. | Integrate AMR awareness into school and university curricula. | Ministry of Federal Education and Professional Training (MoFEPT), MoNHSRC | Educational materials, seminars, curriculum updates | WHO, UNDP, DAI | Annual |
| 10. | Collaborate with ministries to develop and promote policies on pharmaceutical waste and antibiotic use. | MoCC, MoNHSRC, MoIP | Policy briefs, meetings, workshops | WHO, FAO, UNDP | Bi-annual |
| 11. | Share research findings on AMR in environmental compartments. | MoNHSRC, PCRWR | Reports, conferences, publications | WHO, DAI, PCRWR | Bi-annual |
| 12. | Establish partnerships with local NGOs for grassroots AMR awareness campaigns. | MoNHSRC, Local NGOs | Community outreach programs, awareness campaigns | WHO, Local Community Groups | Quarterly |
| 13. | Introduce reward programs for organizations implementing AMR-reducing practices. | MoNHSRC | Awards, recognition programs, public announcements | WHO, Local Business Associations | Annual |
| 14. | Participation in International AMR Forums | Ministry of Health, MoNFSR, MoCC | Conferences, forums, reports | WHO, FAO, WOAH, UNEP | Annually |
| 15. | Adoption of International Best Practices | Ministry of Health, MoCC, MoNFSR | Workshops, partnerships, guidelines | WHO, UNEP, FAO, WOAH | Ongoing/Continuous |
| 16. | Harmonizing National Standards with International Guidelines | MoNHSRC, MoCC, MoNFSR | Policy documents, workshops, reports | WHO, FAO, UNEP | Ongoing/ Annual Reviews |
| 17. | Training Programs | MoNHSRC, MoNFSR Environmental Protection Agency | Hands-on Lab Training Workshops, Seminars | WHO, FAO, OIE, National Research Institutes | Annually or as required |
| 18. | Fellowship and Exchange Programs | MoST, Higher Education Commission (HEC) | Formal Agreements, Memorandums of Understanding (MoUs), Direct Communication | International Centers of Excellence, WHO Collaborating Centers | Annually or as opportunities arise |

| Sr # | Activity | Key Responsible Organizations | Mode of Communication | Supporting Organizations | Frequency |
|---|---|---|---|---|---|
| 19. | Specialized leadership training on AMR policies, stewardship, and surveillance | MoNHSRC, MoNFSR, MoCC | Workshops, seminars, leadership training programs | WHO, FAO, CDC | Annually or as required |
| 20. | Capacity Building on AI/ML for Predictive Modeling and Trend Analysis | MoNHSRC, Pakistan Environmental Protection Agency (PEPA) | Workshops, training sessions, webinars | International AI/ML experts, University partnerships | Annually or as required |
| 21. | Training for Government Agencies and Laboratories | MoNHSRC, Pakistan Environmental Protection Agency (PEPA) | Workshops, Seminars, Webinars | International AI/ML experts, University partnerships | Annual/As needed |
| 22. | Developing AI/ML Infrastructure | MoNHSRC | Official Documentation, Meetings | Local IT/Tech Firms, International Research Institutions | One-time setup, periodic reviews |
| 23. | Integration of AI/ML in Surveillance Networks | PCRWR Provincial Health Authorities | Online Platforms, Workshops | WHO, CDC, AI/ML research groups | Ongoing, with annual updates |
| 24. | Data Quality and Standardization | PEPA, PCRWR | Official Documentation, Workshops | National Data Management Institutes, WHO | Annual |

## 2.19 Research and Development (R&D) Plan for AMR Surveillance in Environment in Pakistan

The following R&D Plan outlines a comprehensive strategy to strengthen the surveillance of Antimicrobial Resistance (AMR) in environmental settings across Pakistan. This plan focuses on enhancing monitoring infrastructure, adopting advanced surveillance tools, implementing innovative data collection methods, fostering cross-sector collaboration, and ensuring the operational effectiveness of surveillance systems. The overarching goal is to support evidence-based decision-making for AMR control and improve public health interventions.

## 2.20 Key R&D Initiatives

- Broaden the scope of AMR surveillance by increasing sentinel sites in both urban and rural areas.
- Develop platforms for seamless sharing of information across human, animal, and environmental health sectors.
- Create tools for the quick detection of AMR pathogens in environmental samples.
- Establish Next-Generation Sequencing (NGS) facilities to identify resistance genes and track the spread of AMR.

- Develop tools tailored for rural and resource-limited settings to enhance local surveillance capacity.
- Leverage AI for analysing AMR data and predicting resistance trends.
- Utilize mobile health technologies for real-time data collection and reporting from remote areas.
- Promote a One Health approach to connect human, animal, and environmental health sectors in AMR surveillance.
- Forge public-private partnerships to expand data collection capacity and foster collaboration across sectors.
- Conduct operational research to identify gaps in current AMR surveillance systems and develop new methods for improvement.
- Support implementation research to overcome barriers to adopting AMR surveillance technologies, especially in resource-limited areas.
- Investigate and optimize wastewater treatment technologies to reduce AMR contamination in wastewater.
- Explore decentralized wastewater solutions for rural and peri-urban areas to mitigate AMR spread.
- Use R&D findings to provide policy and regulatory guidance on AMR environmental surveillance.
- Disseminate research findings to policymakers, healthcare providers, and the public to promote informed decision-making and effective resource allocation.

This R&D strategy focuses on creating innovative solutions to enhance environmental AMR surveillance, improve data collection and analysis systems, and foster cross-sector collaboration. By addressing the need for advanced technologies, public-private partnerships, and comprehensive policy guidance, this plan aims to reduce AMR contamination and mitigate its spread in environmental settings.

## 2.21 KPIs for AMR Surveillance in Environment

Key Performance Indicators (KPIs) are essential for effectively monitoring and evaluating the progress of AMR environmental surveillance, ensuring that interventions are data-driven, measurable, and aligned with national and global health objectives. This table can be used as a practical framework to implement and monitor AMR environmental surveillance, with specific, measurable targets to gauge success and make improvements over time.

**Table 9: KPIs for AMR Environment Surveillance**

| KPI | Description | How to Gauge | Target |
|---|---|---|---|
| **Antibiotic Residue Levels in Environmental Samples** | Percentage reduction in antibiotic residues in water and soil samples from high-risk areas (e.g., healthcare facilities, agricultural zones, wastewater treatment plants) | Regular environmental sampling and testing for antibiotic residues, comparing results over time | Reduction of specific antibiotic residues by 20% annually |
| **Detection of AMR Bacteria in Environmental Samples** | Percentage of environmental samples (water, soil, effluent) testing positive for | Conduct regular environmental sampling at predefined hotspots and monitor the prevalence of | Reducing the percentage of resistant bacteria found in high-risk |

| KPI | Description | How to Gauge | Target |
|---|---|---|---|
| | resistant bacteria or resistance genes | resistant bacteria (e.g., Escherichia coli, Klebsiella) and resistance genes (e.g., bla genes) | areas by 15% annually |
| **Surveillance Coverage** | Percentage of targeted regions or sectors (healthcare, agriculture, wastewater, etc.) with regular AMR surveillance | Track the number of surveillance stations, regions, and sectors covered by environmental AMR surveillance programs | Achieve 100% coverage in high-risk areas by year five of the program |
| **Antimicrobial Use in Agriculture and Veterinary Sectors** | Reduction in the volume of antibiotics used in agriculture and veterinary sectors for non-therapeutic purposes (e.g., growth promotion) | Monitor sales data, prescription data from veterinary clinics, and reports from farms | Achieve a 10% reduction in non-therapeutic antimicrobial use annually |
| **Pharmaceutical Waste Management Compliance** | Percentage of pharmaceutical and healthcare waste treated or disposed of according to environmental guidelines | Track the amount of pharmaceutical waste being properly disposed of or treated using approved methods | 90% compliance with pharmaceutical waste disposal regulations in healthcare and pharmaceutical facilities |
| **Public and Stakeholder Engagement** | Number of public awareness campaigns, workshops, or training programs conducted on AMR environmental issues | Track the number of outreach events held and the attendance/engagement of targeted stakeholders (farmers, healthcare workers, regulatory bodies) | Conduct at least 20 public engagement activities annually, reaching a minimum of 500 stakeholders each |
| **Infection Control and Wastewater Treatment Improvements** | Number of healthcare facilities or farms implementing improved infection control or wastewater treatment practices to reduce AMR | Monitor the implementation of best practices in wastewater treatment, sterilization protocols, and infection control at healthcare and agricultural sites | 80% of healthcare facilities and 60% of farms should adopt improved practices within two years |
| **AMR Data Availability and Reporting** | Timeliness and quality of AMR environmental data reporting to national and international health organizations | Evaluate the accuracy, completeness, and timeliness of environmental AMR data submission to regulatory bodies and international organizations | Achieve a 95% on-time reporting rate for AMR environmental data |
| **Development of AMR Action Plans** | Number of AMR-focused action plans developed or revised based on environmental surveillance findings | Track the creation or revision of national, regional, or sector-specific AMR action plans that integrate findings from environmental surveillance | Develop or revise action plans for at least 3 sectors or regions annually |

## 2.22 Phased Approach to Expand Surveillance Capacity

To address AMR effectively, surveillance systems will be expanded and optimized focusing on:

- **Phase 1:** Initial assessment and enhancement of current surveillance capabilities in key sectors.
- **Phase 2:** Expansion to include more geographic areas, sectors, and environmental media.
- **Phase 3:** Integration of advanced technologies such as AI/ML for predictive modelling, trend analysis, and decision support.

## 2.23 Timeline and Funding Opportunities

The costs associated with AMR surveillance are substantial, requiring significant financial resources. To implement and sustain AMR surveillance activities in Pakistan, funding will be sourced from both domestic and external avenues. The Government of Pakistan, through relevant ministries such as the Ministry of National Health Services, Regulation, and Coordination (MoNHSRC), Ministry of Climate Change (MoCC), Ministry of Water Resources (MoWR), and Ministry of National Food Security & Research (MNFS&R), is expected to allocate funds for these initiatives.

A significant portion of the funding is anticipated to come from Pakistan's Annual Development Plans (ADP) and Public Sector Development Plans (PSDP), with lead organizations responsible for mobilizing resources. Additional financial support will be sought from international development partners and donors focused on AMR, such as FAO, WHO, OIE, World Bank, UNDP, and other global, regional, and country-level AMR programs. Other global partners, including The Fleming Fund, GARP, ReAct, UNEP, and bilateral partners like USAID, FCDO, and WHO, can also contribute to financing AMR surveillance activities in Pakistan. These funding sources will play a crucial role in enhancing Pakistan's capacity to combat AMR by improving surveillance, research, and the implementation of mitigation strategies.

The sectoral departments under the MoNHSRC, MoCC, MoWR, and MNFS&R will carry out their responsibilities as outlined in the strategy, in line with their governance mechanisms and rules of business. They will collaborate closely with provincial and district-level authorities to ensure the successful execution of the surveillance strategy. This collaborative approach will ensure efficient resource use, comprehensive coordination, and integration of AMR surveillance across multiple sectors.

## 2.24 Monitoring and Periodic Review Process

The environmental AMR surveillance strategy is structured for a 3-year period, with inherent flexibility to adapt to evolving developments and critical changes in the field. This dynamic approach is supported by a Monitoring, Evaluation, and Learning (MEL) framework, facilitating periodic reviews at key intervals, such as biannual and annual check-ins. These reviews will assess progress, identify emerging challenges, and recalibrate strategies as necessary. Key elements of the review process include:

- **Annual Review Meetings** will be convened by multi-sectoral AMR committees to assess progress, discuss MEL findings, and address any emerging issues in AMR management. These reviews will incorporate data trends and feedback from field reports.

- **Biannual Field Assessments** will be conducted to evaluate the practical application of AMR surveillance practices, including the monitoring of environmental samples and intervention effectiveness in various settings.

- **Midterm and Final Evaluations** will be carried out to comprehensively assess the long-term impacts of the AMR surveillance strategy, capturing best practices and evaluating overall program effectiveness. These evaluations will inform decisions on program adjustments, expansion, and future initiatives.

# 3 Proposed Framework for a Pilot Study on AMR Surveillance in Environment in Pakistan

## 3.1 Rationale

Antibiotic overuse across various sectors in South Asia, particularly in countries like Pakistan, has led to significant environmental contamination and exacerbated antimicrobial resistance (AMR), posing severe public health challenges. Without intervention, routine infections could become fatal, as seen in Europe, where AMR causes approximately 33,000 deaths annually (Chandra et al., 2021, Fongang et al., 2023). In Pakistan, easy access to antibiotics without prescriptions and the absence of standard treatment guidelines have led to widespread resistance, notably to common antibiotics like fluoroquinolones and sulphonamides (Suzuki and Hoa, 2012). A study revealed that 82% of Escherichia coli isolates from private hospitals were resistant to beta-lactam antibiotics, with many also resistant to fluoroquinolones and trimethoprim-sulfamethoxazole due to overuse (Thiong'o, 2012). The lack of appropriate antimicrobial therapy, insufficient surveillance, inadequate regulation in human and agricultural use, and the absence of wastewater treatment in pharmaceutical companies further exacerbate AMR, highlighting the need for urgent action (Kotwani et al., 2021).

The overuse of antibiotics in South Asia, particularly Pakistan, has led to significant environmental contamination and worsened antimicrobial resistance (AMR), posing public health risks. Environmental AMR surveillance is crucial to identify resistant pathogens in ecosystems and their transmission pathways, enabling targeted interventions and aligning with One Health strategies. In Pakistan, where healthcare access and water treatment facilities are limited, this surveillance is vital for addressing waterborne AMR infections and improving public health outcomes. A pilot case study within the strategy will monitor AMR in water and wastewater sources, assess public health risks, and inform targeted interventions. Water bodies are key reservoirs for resistant pathogens, making water monitoring a more effective and practical approach than soil or air for detecting AMR trends and enabling real-time assessments of human and animal impacts. Considering this advantage, current pilot study is proposed to be focused on the AMR monitoring of water and wastewater sources.

## 3.2 Study Aim and Objectives

Given the pressing need for effective action against AMR and its environmental implications, this pilot study aims to conduct a comprehensive investigation to inform the development of a policy document for the Pakistani government. This document will advocate for stringent enforcement of environmental regulations aimed at protecting human and environmental health from antibiotic residues and associated AMR challenges. Specific objectives are as following:

- **Strengthen Capacity for AMR Surveillance:** Enhance the technical skills of the workforce by providing advanced training in AMR surveillance, including sample collection, microbiological analysis, genomic sequencing, and data management to ensure comprehensive and systematic environmental monitoring.
- **Reconnaissance Survey for designing the AMR Surveillance Survey:** Reconnaissance survey will be undertaken in the study area to pinpoint the high-risk areas for sampling, including locations impacted by disposal of municipal and commercial wastewater

effluents, livestock waste, hospital waste, poultry waste, industrial wastewater, solid waste dumping sites etc.

- **Assess Environmental Transmission Risk of Key AMR Pathogens:** Monitor and evaluate the transmission pathways of Salmonella, Vibrio cholerae, and E. coli across environmental matrices mainly water on monthly basis up to 12 months to understand their ecological persistence and potential risks to public health.
- **Assess Antibiotic Susceptibility of AMR Isolates:** To assess the antibiotic susceptibility of environmental isolates by conducting antimicrobial susceptibility testing using methods such as disk diffusion and Vitek 2, enabling the identification of resistance patterns and informing targeted antimicrobial interventions.
- **Perform Genomic Characterization of AMR Isolates:** To perform genomic characterization of antimicrobial-resistant isolates through techniques like whole genome sequencing, facilitating the identification of resistance mechanisms, tracking gene transfer, and enhancing the understanding of AMR dynamics in environmental contexts.
- **Establish Baseline Data and Advocate for Regulatory Reforms:** Generate baseline data on the prevalence of AMR contaminants in environmental sources, using both conventional and genomic tools. Leverage these insights to promote evidence-based policy interventions, including stricter controls on antibiotic use and over-the-counter drug sales, ensuring the protection of public health and ecosystems.

## 3.3 Scope of the Pilot Study

In Islamabad, the availability and quality of water resources are essential for meeting the needs of urban and rural populations. The Capital Development Authority (CDA) manages major water supplies through Simly Dam (24 MGD) and Khanpur Dam (7 MGD), while approximately 45% of the city's water comes from 200 tubewells (28 MGD). Despite a population increase from 1.01 million in 2017 to 1.232 million in 2023, the water supply remains inadequate, with a demand of 120 MGD compared to a peak supply of 62.70 MGD (CDA, 2022). In addition to surface and groundwater water supply sources, Islamabad and Rawalpindi are home to several natural streams that play a crucial role in the region's water systems, though many are vulnerable to pollution from untreated wastewater and solid waste. These natural streams include:

1) **Shahdara Stream:** At upstream, Low contamination, originating from Margalla Hills (reference site) and at Downstream it passes through Bhara Kahu, carrying poultry and livestock waste; joins the Korang River. Also impacted by tourism activities.
2) **Trail-3 Stream**: Originates from the Margalla Hills and collects municipal waste from Sectors F-5 and F-6 and hospital waste near Polyclinic Hospital.
3) **Gumrah Kas (Chatta Bakhtawar)**: Passes through residential areas, carrying solid waste.
4) **Gumrah Kas (Tarlai Kalan)**: Carries poultry farm waste, livestock waste, and agricultural runoff.
5) **Lai Nullah (Lai Stream)**: A significant drainage channel collecting wastewater and runoff from Islamabad and Rawalpindi, often causes floods during the monsoon season.

6) **Korang River**: Originates from the Murree Hills and feeds Rawal Dam.
7) **Rawal Lake Inflows (Korang Tributaries):** Smaller streams that feed Rawal Dam, prone to contamination from settlements and agriculture.
8) **Soan River:** A major river flowing through Rawalpindi, receiving storm water and untreated sewage, eventually joining the Indus River.
9) **Simly Dam Inflows & Nurpur Stream**: Streams from nearby hills that feed Simly Dam (Islamabad's second major water source). The Nurpur Stream, flowing through rural Islamabad, is vulnerable to waste dumping and agricultural runoff.

Many of these streams originate from Margalla Hills, flow through Islamabad's residential sectors, and carry domestic, hospital, and industrial waste along with storm water. These along with many tributaries then enter Rawalpindi as the Lai drainage channel, eventually joining the Korang River and draining wastewater into the river near Soan Bay. This wastewater mixing and groundwater seepage are expected to further deteriorate both surface and groundwater quality. These natural streams serve as both water sources and drainage channels but are under increasing pressure from urbanization, untreated wastewater, agricultural runoff, and commercial activities. Possible sources of AMR include the disposal of untreated wastewater, agricultural runoff containing antibiotics, and contamination from healthcare facilities. The growing population and urbanization, particularly during the summer and pre-monsoon seasons, highlight the urgent need for AMR environmental surveillance in the Islamabad-Rawalpindi area. Monitoring AMR is crucial to identify and mitigate the risks posed by resistant pathogens in water supplies, thereby protecting public health and ensuring safe water resources for the community. Monitoring these streams for AMR is essential to assess public health risks and manage water quality effectively in the Islamabad-Rawalpindi region. Following this, the current study proposal aims to provide actionable insights to help decision-makers address pressing water challenges and strengthen Islamabad's water systems against future uncertainties while focusing on AMR surveillance to mitigate the risks of antibiotic-resistant pathogens in the water supply.

## 3.4 Study Implementing Agency

This proposed pilot study on AMR surveillance in environmental sources is crucial for understanding the local AMR landscape and its implications for public health. This pilot study is proposed to be implemented by the Pakistan Council of Research in Water Resources (PCRWR), an apex body under the Ministry of Water Resources and the only federal-level research organization dedicated to water management. PCRWR is mandated to conduct, organize, and promote research across all aspects of water resources, including irrigation (surface and groundwater), drainage, soil reclamation, drinking water, and rainwater harvesting. Headquartered in Islamabad, PCRWR operates eight regional offices in Lahore, Karachi, Quetta, Bahawalpur, Peshawar, Tandojam, Gilgit, and Muzaffarabad, along with 24 water quality testing laboratories and 8 research and demonstration farms. The National Water Quality Laboratory at its Islamabad headquarters, accredited to ISO-17025 and certified by the EPA, provides analytical services covering a broad range of parameters, including microbiological, inorganic, organic, and persistent organic pollutants (POPs) for drinking, irrigation, and industrial applications.

PCRWR plays a pivotal role in national water quality monitoring programs, environmental assessments, and AMR surveillance. It has spearheaded the rehabilitation of traditional water systems like Karezes, enhanced WASH (Water, Sanitation, and Hygiene) infrastructure in

schools, and led relief efforts during cholera outbreaks and floods. Additionally, PCRWR manages constructed wetlands for wastewater recycling and regularly monitors the quality of bottled water to safeguard public health. Collaborating with partners such as the National Institute of Health (NIH), PCRWR has undertaken initiatives like the Extended Tricycle Project, which focuses on detecting carbapenem-resistant *E.coli* through monthly sampling from rivers, wastewater, and wet markets in Islamabad, Lahore, and Khyber Pakhtunkhwa. These samples undergo microbial culture and antimicrobial sensitivity testing to monitor resistance trends. Through national and international partnerships, PCRWR actively contributes to sustainable water management, the achievement of SDG 6 targets, and the prevention of waterborne diseases across Pakistan.

PCRWR in 2024 (January, June, July, & October 2024) have undertaken a comprehensive mapping of water resources mapping to understand availability, manage resources, identify vulnerable areas, conserve the environment, and plan for climate change impacts. This was achieved through delineation of urban and rural areas of Islamabad, mapping of natural streams, dams and tube wells of Islamabad, flow measurement; chemical and biological water quality assessment of all streams, river, as well as surface and ground water supplies of Islamabad (*Figure-7*). With an established monitoring history and data, PCRWR is well-positioned to execute the proposed pilot study effectively adopting following methodology.

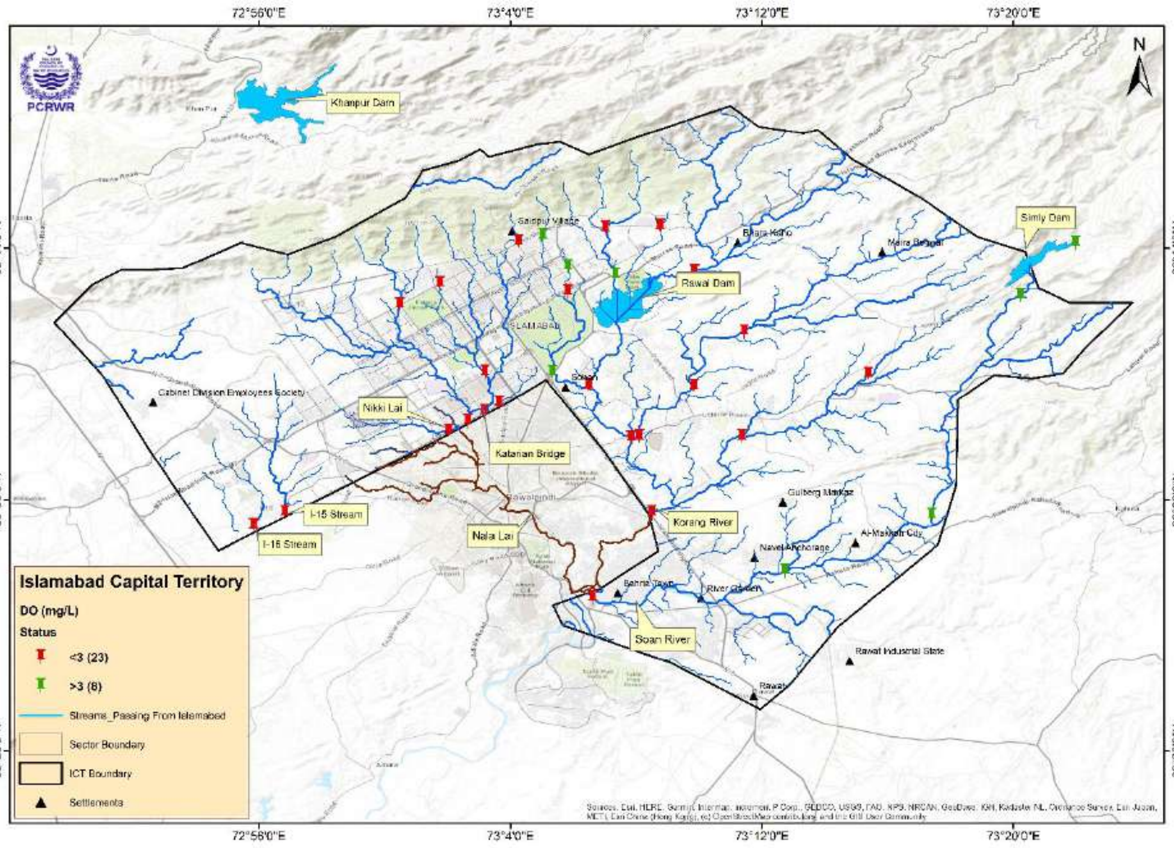


*Figure 7: Main surface water streams of Islamabad (Source: PCRWR, 2024)*

## 3.5 Methodology

To achieve above listed objectives, a methodology is described here to follow a multi-step approach to align with the study objectives, including environmental sampling, microbiological analysis, antibiotic susceptibility testing, genomic characterization, and data-driven policy recommendations.

**Objective-1:** Strengthen Capacity of Water Quality Laboratories for AMR Surveillance

To strengthen the capacity for AMR surveillance, PCRWR with support of DAI and Fleming's funds will implement a comprehensive approach focused on enhancing the technical skills of its workforce. This includes organizing regular workshops and hands-on training sessions led by experts in AMR surveillance, microbiology, and genomics, covering essential topics such as sample collection, microbiological analysis methods, and genomic sequencing protocols. PCRWR will develop Standard Operating Procedures (SOPs) to ensure consistency and quality across all operations, accompanied by training on their implementation. Investment in modern laboratory equipment and a dedicated budget for on-going education will further empower staff. Monitoring and evaluation will be integral, with established performance metrics to assess the effectiveness of training programs and feedback mechanisms to refine content continuously. By focusing on these strategies, the pilot aims to cultivate a skilled workforce capable of conducting systematic AMR surveillance, ultimately contributing to improved public health outcomes and effective management of antimicrobial resistance in environmental sources.

### 3.5.1 AMR Surveillance Survey Design

**A. Sampling Frame:**

The sampling frame for AMR surveillance in Islamabad-Rawalpindi will be based on the comprehensive mapping earlier conducted by the PCRWR in 2024, encompassing 09 natural streams (altogether 31 including tributaries), watersheds of major water bodies such as Simly Dam and Rawal Dam, and 200 active tubewells. This frame will include specific sampling sites along natural streams, focusing on areas where flow measurements were taken, and highlighting potential contamination sources such as the disposal of untreated municipal wastewater, cattle farming, livestock operations, solid waste dumping sites, hospital wastewater, poultry waste, and unregulated commercial activities. Additionally, the frame will incorporate historical water quality assessment locations, prioritizing those with previous records of antimicrobial resistance. By delineating possible AMR sources, the sampling frame will ensure a systematic approach to monitoring AMR, facilitating effective data collection across diverse water resources while leveraging PCRWR's established history of monitoring and analysis for enhanced public health and environmental protection.

**B. Sampling Method**

A random sampling method is selected to ensure representative coverage across diverse environmental conditions, minimize selection bias, and capture variations in water quality and potential AMR hotspots.

A sample size from 09 natural streams is calculated using Cochran formula (Equation. 1):

$$n_o = \frac{Z^2 \times \rho\,(1-\rho)}{e^2}$$

$n_o$ = estimated sample size
Z= the critical value obtained from a standard normal distribution. For each level of confidence there is a corresponding value of z. (95%: corresponding z value of 1.96)
p= estimated prevalence of AMR contamination in the study area (prevalence of 0.5)
e=margin of error at 5% (standard value of 0.05).

$$n_o = \frac{(1.96)^2 \times 0.5\,(1-0.5)}{(0.05)^2}$$

$$n_o = 384.16$$

Calculations showed a sample size of 384.16. After calculating the initial sample size as above, the **finite population correction** in accordance with the Equation-2 is applied:

$$n = \frac{n_o}{1 + \frac{(n_o - 1)}{N}}$$

Where $N$ is the total number of units (streams, dam points, and tubewells) in the sampling frame.

$$n = \frac{384.16}{1 + \frac{(384.16 - 1)}{9}}$$

$$\boldsymbol{n = 10}$$

**C. Sampling Frequency:**

Each selected site will be sampled monthly for 12 months to monitor seasonal variations in AMR trends. This will provide robust data on how environmental factors (e.g., rainfall, temperature) influence the presence and spread of AMR.

### 3.5.2 Reconnaissance Survey

PCRWR's four-member technical team has conducted a comprehensive reconnaissance survey across Islamabad-Rawalpindi to identify key sites for representative AMR surveillance. The survey included physical assessments of catchments and upstream and downstream sections of major surface water bodies to pinpoint potential AMR sources, such as untreated municipal wastewater, livestock effluents, solid waste dumping sites, hospital and poultry waste, unregulated commercial activities, and discharges from pharmaceutical industries.

To effectively monitor the environmental transmission of antimicrobial-resistant microorganisms, PCRWR will implement a systematic sampling approach covering these diverse contamination sources. The strategy will also focus on the inflow and outflow points of wastewater treatment plants, alongside groundwater sampling along streams, drains, and riverbanks to assess AMR infiltration through seepage.

Based on the survey, seven key sources have been identified for monthly sampling over a 12-month period, as detailed in *Figure-8* and *Table-10*. Sites will be strategically selected near pollution entry points, including hospital discharges, pharmaceutical effluents, and residential zones. Sampling will target surface water bodies, groundwater sources, and drains that transport effluent, aiming to evaluate the environmental impact of antibiotic contamination. To capture seasonal variations, samples will be collected during both dry and wet seasons.

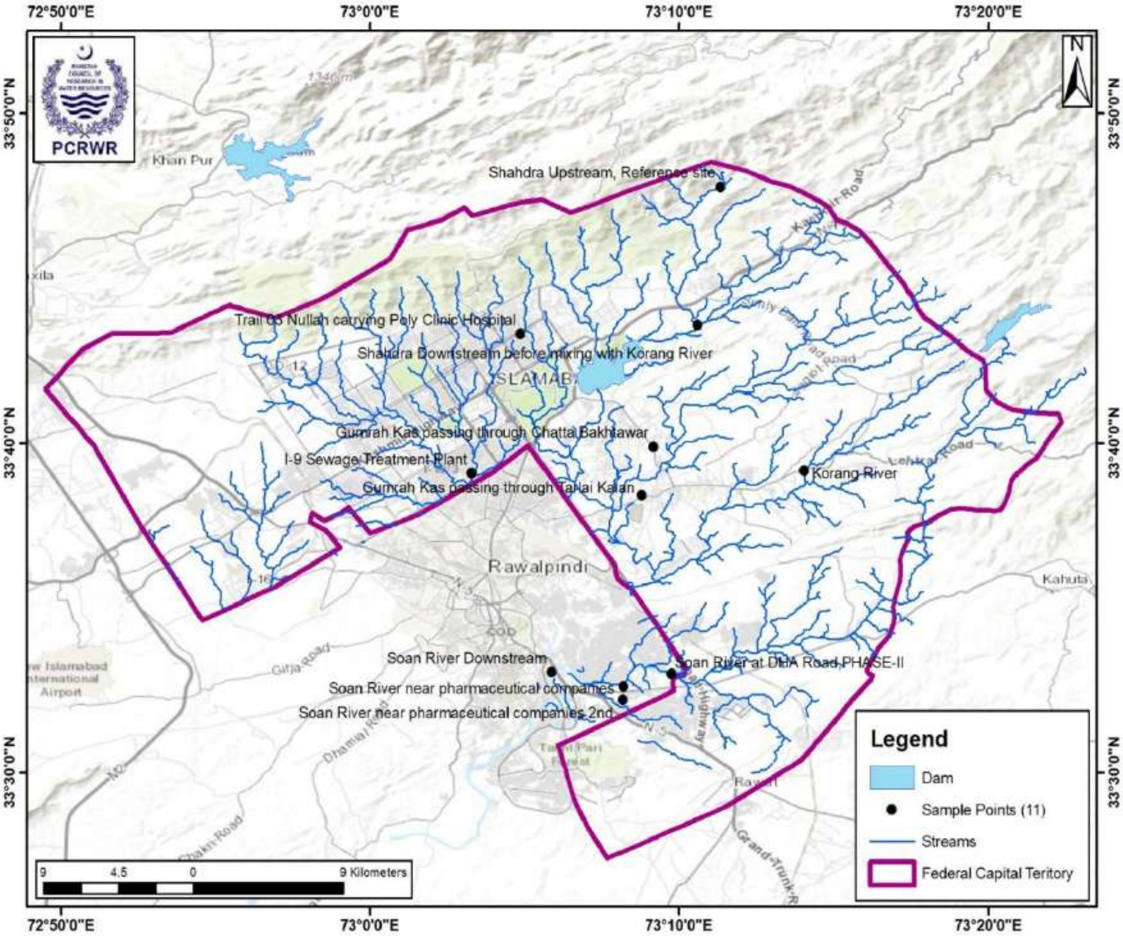


*Figure 8: Map indicating sites selected for AMR Surveillance on monthly basis*

**Table 10: Sampling sites identified in Islamabad-Rawalpindi for AMR Surveillance**

| Sr. No. | Site Name | GPS Coordinates | Sources of Contamination |
|---|---|---|---|
| 1. | Shahdra Upstream (Reference site) | 33°47'45.3"N 73°11'20.7"E | This site has taken as reference site as it originates from Margalla Hills and carrying very low level of contamination |
| 2. | Shahdra Downstream before mixing with Korang River | 33°43'33.6"N 73°10'36.3"E | Shahdra downstream passing through the Bhara Kahu city carrying **Poultry and livestock wastes** and falls into the Korang River near Abbasi Town Dhok Jilani |
| 3. | Trail-3 stream near Polyclinic Hospital | 33°43.298"N 073°004.881"E | Trail-3 Stream originates from the Margalla Hills carries the municipal waste of sectors F-5 and F-6 and later mixes into the drain located near Polyclinic Hospital and carrying **Hospital waste.** |
| 4. | Stream Gumrah Kas: Course passing through Chatta Bakhtawar | 33º39’52.591"N 73º9’10.8573"E | Gumrah Kas near Chatha Bakhtawar carrying **Solid Waste** of the residential settings. |

| Sr. No. | Site Name | GPS Coordinates | Sources of Contamination |
|---|---|---|---|
| 5. | Stream Gumrah Kas: Course passing through Tarlai Kalan | 33.6399573"N 73.1467034"E | Gumrah Kas carries the wastes of **Poultry** (new Anmol chicks and Marwat Farm house, Tarlai vegetable and poultry farms); and **Livestock Farm** house (Haq Foods, Alanafay Goat Farm, Qureshi Cattle Farm) |
| 6. | I-9 Sewage Treatment Plant (at outlet) | 33.651054"N 73.054995"E | The Sewage Treatment plant carrying the **Municipal and Industrial wastes** of residential sectors of Islamabad and I-9 industrial area. Due to inadequate treatment capacity, most of the collected wastewater is inadequately treated and discharged into the Lai Nullah. |
| 7. | Korang River near Sihala Al Makkah city | 33.568218N, 73.256836E | Korang river at Sihala bridge **Municipal waste** |
| 8. | Soan River site near pharmaceutical companies | 33.543232"N 73.136747"E 33.543293"N 73.136432"E | About 12 to 15 **Pharmaceutical Companies** (Paramount, IPP, Innvotek etc.) located near the Soan River possibly carrying pharmaceutical waste into river at this point. |
| 9. | Soan River Downstream | 33°33'02.2"N 73°05'53.2"E | Municipal, Livestock and Industrial wastes of Rawat Industrial Estate |
| 10. | Soan River at DHA Road, DHA Phase-II | 33°32'58.2"N 73°09'45.9"E | DHA Phase II Nullah carrying **Slaughter House waste** and drains into the Soan River |

### 3.5.3 Selection of E. coli, Salmonella, and Vibrio cholerae for AMR Surveillance

These bacteria are frequently detected in surface waters and are known etiological agents of waterborne infections, with antimicrobial-resistant (AR) strains identified globally in both developed and developing countries. Despite their significance, data on *Salmonella*, *E. coli*, and other pathogens such as *Enterococcus* in environmental water are limited. To better understand their development, prevalence, and dissemination, it is essential to adopt the One Health Approach, which integrates human, animal, and environmental health perspectives. Monitoring the prevalence and distribution of these bacteria in surface water, along with identifying their potential sources, will provide critical insights into their impact.

AR bacteria in natural watersheds are poorly studied, and their potential effects on human health, food safety, and ecosystem balance remain unclear. As surface waters act as both a repository and transmission pathway for AR bacteria from human, animal, and industrial sources, addressing this data gap is vital to mitigating the spread of resistance.

For this surveillance, *Salmonella*, *E. coli*, and *Vibrio cholerae* were selected due to their roles as both primary and opportunistic pathogens, with a focus on tracking AMR trends in environmental water. Previous studies worldwide highlight the widespread presence of pathogenic and AR bacteria in surface waters, reinforcing the importance of environmental monitoring. Given that surface waters serve as reservoirs for AR bacteria and play a critical role in their dissemination, studying these pathogens will provide essential data for developing strategies to control emerging resistance.

### 3.5.4 Monitor the Environmental Transmission Risk of Key AMR Microorganisms

Standard Operating Procedure (SOP) for Sampling, Detection, and Antibiotic Susceptibility Testing for Salmonella, Vibrio cholerae, and E. coli will be developed as complete detail in the form of "AMR Surveillance Manual for the Environmental laboratories". However, adhering to international protocols for AMR bacteria sampling and testing (e.g., American Public Health Association, 2017), water samples will be collected following the samples collection method (9060, APHA 2017) analysed for the presence and concentration of AMR microorganisms, specifically targeting key pathogens such as Salmonella, Vibrio cholerae, and *E. coli* as listed below:

- E-coli
  - Method No. 9222 Membrane Filter Technique for Members of the Coliform Group (American Public Health Association, 2023);
  - WHO integrated global surveillance on ESBL-producing E. coli using a "One Health" Approach: implementation and opportunities. Geneva: World Health Organization; 2021. License: CC BY-NC-SA 3.0 IGO.
- Salmonella spp.
  - Method No. 9260, Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 2023).
- Vibrio cholera
  - Method No.9260, Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 2023).

In addition to above listed microorganisms, tests such as Biochemical oxygen demand, dissolved oxygen and chemical oxygen demand will also be performed for correlational analysis. To ensure the integrity of water and wastewater sampling for AMR surveillance, sterile containers will be utilized for collecting both water and soil samples, thereby minimizing the risk of contamination. Upon collection, samples will be stored at 4°C to preserve microbial viability and prevent degradation of the samples. It is imperative that all samples are transported to the laboratory within 24 hours of collection to facilitate timely analysis and accurate detection of AMR organisms. This stringent sampling protocol is crucial for obtaining reliable data on the presence of antimicrobial-resistant pathogens in environmental water and wastewater, ultimately contributing to informed public health strategies and effective management of water quality.

Following the isolation of target organisms, serological and/or biochemical confirmations, PCRWR laboratories will conduct phenotypic characterization of *Salmonella, E. coli, and Vibrio cholerae* on Vitek-2 to provide comprehensive data on AMR prevalence.

### 3.5.5 Antibiotic Susceptibility Testing (AST)

The antimicrobial resistance (AMR) of *Salmonella, E. coli, and Vibrio chole*rae will be demonstrated using the disk diffusion technique to determine their resistance profiles and calculate the microbial resistance index of the antibiotics tested, including their potential to produce specific enzymes. The disk diffusion method will be conducted following the Clinical and Laboratory Standards Institute (CLSI) guidelines to ensure standardized and reliable results. Antibiotics commonly used in Pakistan will be employed in the assessment, including nalidixic acid, ampicillin, chloramphenicol, gentamicin, tetracycline, trimethoprim-

sulfamethoxazole, ciprofloxacin, colistin sulfate, ceftazidime, and cefotaxime. To ensure accuracy, quality control strains of three organisms will be included in every test run. During the evaluation, intermediate resistance will be interpreted as resistant to account for clinical relevance. Isolates exhibiting resistance to at least three different antibiotic classes will be classified as multidrug-resistant (MDR), highlighting the severity of the resistance. This method will provide essential data on the AMR profiles of environmental isolates, informing targeted interventions and monitoring trends in antimicrobial resistance.

### 3.5.6 VITEK 2 Characterization

The VITEK 2 automated identification and susceptibility testing system will be employed for the further characterization of isolated strains of *E. coli, Salmonella,* and *Vibrio cholerae*. This system utilizes VITEK 2 GN cards specifically designed for the identification of Gram-negative bacteria, allowing for efficient and accurate biochemical characterization. Through this process, the antibiotic susceptibility profiles of the isolated strains will be determined, providing critical insights into their resistance patterns. The results obtained from the VITEK 2 system will be analysed to confirm the biochemical characteristics of each isolate and evaluate their resistance to a comprehensive panel of antibiotics tailored to the specific pathogens being studied. This characterization will enhance our understanding of the antimicrobial resistance dynamics of E. coli, Salmonella, and Vibrio cholerae, informing targeted treatment strategies and public health interventions.

### 3.5.7 Genomic Analysis

Isolates of confirmed *Salmonella, E. coli,* and *Vibrio cholerae* on monthly basis will *be* transported to the National Institute of Health (NIH) by placing them in sterile containers having ice packs and appropriate packaging to maintain a stable temperature during transport. In NIH, the genomic analysis of confirmed *Salmonella, E. coli,* and *Vibrio cholerae* isolates begins with DNA extraction, where colonies selected from the isolation plates are transferred to sterile micro-centrifuge tubes. A commercial DNA extraction kit will then be employed, following the manufacturer's instructions to isolate genomic DNA effectively for each of the targeted pathogens. Next, the extracted DNA is quantified using a spectrophotometer to ensure it meets the required concentration for sequencing. The library for whole genome sequencing (WGS) is prepared in accordance with the specific protocols of the chosen sequencing platform before submission for sequencing. Following sequencing, bioinformatics tools will be utilized to analyze the generated sequencing data. This analysis focuses on identifying resistance genes and exploring the genetic relationships among the isolates to understand transmission pathways and resistance mechanisms for *Salmonella, E. coli, and Vibrio cholerae*. Furthermore, the genomic data will be compared with existing databases to identify known resistance profiles and mutations, thereby providing valuable insights into the antimicrobial resistance dynamics of these pathogens in environmental contexts.

### 3.5.8 Data Analysis and Results Reporting

For data analysis, statistical software such as SPSS will be utilized to assess prevalence rates of AMR among the isolated pathogens. Additionally, correlation analysis will be employed to identify associations between AMR rates and various environmental factors, such as proximity to sewage disposal sites and agricultural runoff. This comprehensive approach will

enhance our understanding of the dynamics of AMR in the local context and inform targeted interventions for managing resistance.

### 3.5.9 Create Baseline Data and Advocate for Policy Interventions

All findings generated from the PCRWR and NIH labs will be compiled into a comprehensive Technical Report, which will be shared in a consultative workshop with the key stakeholders, including the Ministry of Health, Pakistan's Drug Regulatory Authority, water and environmental organizations, agriculture bodies, chambers of commerce and industry, the Ministry of Water Resources, provincial regulatory agencies, hospitals, and farmers. This report will serve as a critical resource to guide policy development and regulatory actions aimed at mitigating the spread of AMR in water systems. In addition, collaboration with local health authorities, environmental agencies, and academic institutions will be integral throughout the study. Workshops and seminars will be organized to disseminate findings and raise awareness about AMR, fostering a collaborative approach to address this pressing public health issue.

## 3.6 Expected outcomes

Following outcomes are expected from the implementation of this pilot study to significantly contribute to improving public health and environmental sustainability in Pakistan.

1) **Enhanced Public Health and Environmental Protection:** Significant reduction in public health risks associated with AMR in water systems by identifying contamination hotspots and quantifying antibiotic residues to mitigate AMR transmission.

2) **Strengthened Institutional Capacity for AMR Surveillance:** Development of robust infrastructure and training for PCRWR staff, leading to the establishment of a national AMR surveillance framework to support ongoing assessments and evidence-based policymaking.

3) **Support for Sustainable Industrial Practices:** Provision of comprehensive data on AMR contamination to guide industries in adopting sustainable practices, promoting corporate responsibility and compliance with environmental regulations.

4) **Comprehensive Data on AMR Profiles:** Extensive data on the prevalence and antibiotic resistance profiles of *E. coli*, *Salmonella*, and *Vibrio cholerae* in the region.

5) **Identification of Contributing Factors to AMR:** Insights into major contributing factors for targeted public health interventions.

6) **Recommendations for Policymakers:** Actionable recommendations to guide strategies for combating AMR and enhancing water quality management.

## 3.7 Timeline

| Activities | 2024 | | | 2025 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | July | Aug | Sept | Oct | Nov | Dec |
| Stakeholder consultation on the pilot study | | | | | | | | | | | | | | | |
| Preparing SOPs for Sampling, Detection, and Antibiotic Susceptibility Testing for Salmonella, Vibrio cholerae, and E. coli | | | | | | | | | | | | | | | |
| Capacity building of Lab staff on AMR protocols | | | | | | | | | | | | | | | |
| Procurement of consumables, supplies etc. | | | | | | | | | | | | | | | |
| Sample collection from designated sites | | | | | | | | | | | | | | | |
| Laboratory analysis of collected samples, including pathogen isolation. | | | | | | | | | | | | | | | |
| Antibiotic sensitivity testing and phenotypic characterization on Vitek-2 | | | | | | | | | | | | | | | |
| Preservation of Isolates | | | | | | | | | | | | | | | |
| Dispatch of Isolates to NIH for genomic analysis | | | | | | | | | | | | | | | |
| Data analysis and report writing. | | | | | | | | | | | | | | | |
| Dissemination of findings through stakeholder workshops and publications. | | | | | | | | | | | | | | | |

# 4 Risk Assessment and Mitigation Guidelines for Strategy

## 4.1 Introduction

Antimicrobial resistance (AMR) in the environment is an often overlooked but critical factor in the global fight against resistance. Environmental contamination—through pharmaceutical waste, agricultural runoff, and healthcare effluents—serves as a reservoir for resistant pathogens that can spread through ecosystems, impacting human and animal health. Addressing AMR in the environment is essential for protecting public health, ensuring sustainable agricultural practices, and preserving ecosystems. A comprehensive risk assessment and mitigation framework enables countries to effectively monitor, manage, and reduce the environmental spread of AMR.

Environmental AMR surveillance plays a vital role in understanding and mitigating the spread of resistance. By monitoring antibiotic residues, resistant bacteria, and resistance genes, surveillance helps identify hotspots of resistance in the environment and evaluates the effectiveness of interventions such as advanced wastewater treatment and industrial effluent controls. Surveillance supports evidence-based policymaking, informs regulatory changes, and encourages the development of new technologies and strategies to combat resistance. Harmonized surveillance systems also facilitate global comparisons and cross-border cooperation, making it the backbone of a targeted, data-driven strategy to reduce AMR in the environment.

By identifying and assessing key risks, such as the overuse of antibiotics, poor hygiene practices, inadequate waste management, and insufficient monitoring, this framework supports targeted interventions. These interventions include antibiotic stewardship programs, regulated antibiotic use, improved waste treatment technologies, and enhanced sanitation practices. Furthermore, effective surveillance and risk assessment are necessary to detect environmental contamination, identify hotspots, and assess the impact on ecosystems, water quality, and human health. This holistic approach ensures that efforts to combat AMR are comprehensive and effective, helping to safeguard public health, biodiversity, and the sustainability of food production systems. Through cross-sectoral collaboration, environmental AMR surveillance can be strengthened, contributing to the broader global effort to combat AMR.

## 4.2 Risk Assessment and Mitigation Guidelines

Risk assessment and mitigation measures for AMR are critical to addressing its growing threat across human healthcare, veterinary and agricultural sectors, and the environment. Identifying key risks such as overuse of antibiotics, poor hygiene, inadequate waste management, and insufficient monitoring allows for targeted interventions like antibiotic stewardship programs, regulated antibiotic use, and improved waste treatment technologies. Risk assessment for AMR in the environment is particularly important as environmental contamination through pharmaceutical waste, agricultural runoff, and healthcare effluents acts as a reservoir for resistant pathogens, which can spread through ecosystems and affect human and animal health. By identifying hotspots and implementing effective waste management, pollution control, and sanitation practices, we can reduce exposure to resistant bacteria. Environmental risk assessments are a crucial component of a comprehensive AMR strategy, helping safeguard public health, biodiversity, and ecosystem integrity while enhancing global efforts to combat AMR. The following risk assessments and mitigation

strategies address AMR in various sectors, aiming to minimize environmental contamination and safeguard public health.

### 4.2.1 AMR in Human Healthcare

**Key Risks:**

- Overuse and misuse of antibiotics in healthcare settings.
- Inadequate infection prevention and control measures.
- Lack of awareness among healthcare professionals about AMR.

**Mitigation Measures:**

- **Antibiotic Stewardship Programs (ASP):** Ensure proper diagnosis, prescription, and responsible use of antibiotics.
- **Prescription Regulations:** Strengthen laws to ensure antibiotics are dispensed only with valid prescriptions.
- **Infection Prevention and Control (IPC):** Implement stringent infection control practices, including hand hygiene and sterilization.
- **Healthcare Training:** Regularly train healthcare providers on AMR, stewardship principles, and proper antimicrobial use.

### 4.2.2 AMR in Veterinary and Agricultural Sectors

**Key Risks:**

- Unregulated use of antibiotics in livestock for growth promotion and disease prevention.
- Poor animal husbandry practices leading to higher antibiotic dependency.
- Overuse of antibiotics in farming.

**Mitigation Measures:**

- **Regulated Antibiotic Use:** Ensure antibiotics are only used under veterinary supervision and restrict their use for non-therapeutic purposes.
- **Good Animal Husbandry Practices:** Promote practices like improved nutrition, vaccination, and sanitation to reduce antibiotic use.
- **Alternatives to Antibiotics:** Encourage the use of probiotics and immunomodulators to reduce antibiotic dependency in livestock.
- **Farmer Education:** Provide training on the risks of antibiotic misuse and the importance of proper veterinary guidance.

### 4.2.3 Environmental Contamination by AMR

**Key Risks:**

- Pharmaceutical and healthcare waste contaminating water and soil.
- Presence of antibiotic residues and resistant bacteria in the environment.
- Poor waste management practices in healthcare and pharmaceutical sectors.

**Mitigation Measures:**

- **Proper Waste Management:** Establish systems to prevent contamination from antibiotics and resistant bacteria, including wastewater treatment.
- **Environmental Hotspot Surveillance:** Monitor high-risk areas such as water bodies near farms and pharmaceutical plants for antibiotic contamination.
- **Awareness Campaigns:** Raise awareness about the risks of improper pharmaceutical waste disposal and promote adherence to environmental regulations.

### 4.2.4 Technological Limitations in Waste Treatment

**Key Risks:**

- Inadequate technologies to remove antibiotics and resistant bacteria from wastewater and soil.
- Lack of on-site treatment systems for pharmaceutical and healthcare waste.

**Mitigation Measures:**

- **Innovative Waste Treatment Technologies:** Invest in advanced technologies like membrane bioreactors and bioremediation to treat contaminated water and soil.
- **On-Site Pharmaceutical Waste Treatment:** Encourage pharmaceutical manufacturers and healthcare providers to install waste treatment systems capable of breaking down antimicrobial residues.

### 4.2.5 Water, Sanitation, and Hygiene (WASH) Challenges

**Key Risks:**

- Poor sanitation and hygiene in healthcare facilities and communities leading to the spread of AMR.
- Contaminated water sources contributing to the proliferation of resistant bacteria.

**Mitigation Measures:**

- **Strengthen WASH Programs:** Improve water, sanitation, and hygiene practices to reduce the spread of AMR through contaminated water and poor hygiene.
- **Community-Level Interventions:** Implement WASH programs in areas with high antimicrobial usage, especially near healthcare facilities and farms.

## 4.3 Lack of Effective Monitoring and Evaluation (M&E) Framework

**Key Risks:**

- Inadequate monitoring of AMR trends and interventions.
- Poor data collection and lack of standardized methods for tracking AMR in the environment.

**Mitigation Measures:**

- **Key Performance Indicators (KPIs):** Develop measurable indicators to track reductions in antibiotic prescriptions, antibiotic residues, and public awareness.

- **Baseline Data and Targets:** Conduct initial assessments to establish baseline data and set targets for improvement.
- **Data Standardization:** Implement standardized methods for data collection across sectors, using Geographic Information Systems (GIS) to map AMR hotspots.
- **Regular Monitoring and Reporting:** Conduct quarterly or annual reviews to track progress and ensure transparency.
- **Feedback Mechanisms:** Establish feedback loops for stakeholders to share insights and improve strategies.

## 4.4 Global and National Policy Gaps

**Key Risks:**

- Lack of cohesive policies addressing environmental AMR.
- Limited capacity for environmental AMR monitoring in low- and middle-income countries.

**Mitigation Measures:**

- **Advocacy for Environmental AMR in Global Action Plans:** Promote the inclusion of environmental AMR in global frameworks like the Global Action Plan on AMR.
- **International Collaboration:** Build partnerships with global organizations to strengthen environmental AMR surveillance, especially in resource-limited settings.

## 4.5 Conclusion

Addressing environmental AMR requires a multifaceted approach, with surveillance, risk assessment, and mitigation strategies forming the core of any effective response. By implementing the guidelines provided in this chapter, Pakistan can strengthen its environmental AMR surveillance systems, minimize contamination risks, and protect both public health and the environment. This approach will help align national efforts with global standards for sustainable health and environmental management.

## 4.6 Limitations

A significant limitation in developing this AMR environmental strategy was the lack of available AMR environmental data and relevant studies from key departments and agencies. This absence of targeted and reliable data severely hampered the ability to conduct a thorough and detailed analysis of antimicrobial resistance (AMR) within the context of environmental health. Without sufficient data on the prevalence, distribution, and trends of resistant pathogens in environmental sources such as water and wastewater, it becomes challenging to identify key hotspots, monitor resistance dynamics, and assess the effectiveness of existing policies. Furthermore, the lack of coordinated data collection and sharing among relevant institutions limited the scope of the strategy and hindered the development of comprehensive management frameworks. To address these gaps, future efforts must prioritize the establishment of robust AMR surveillance systems, with dedicated research and data collection on environmental AMR from national and local authorities, to inform more effective interventions and policy recommendations.

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